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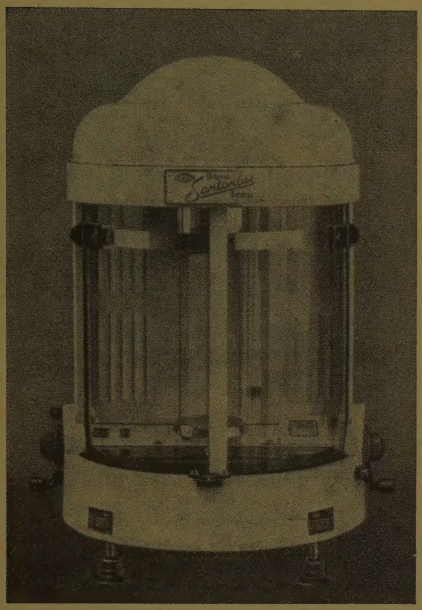

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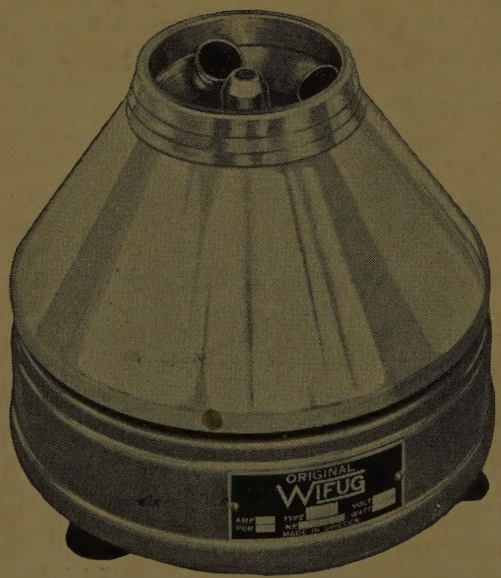

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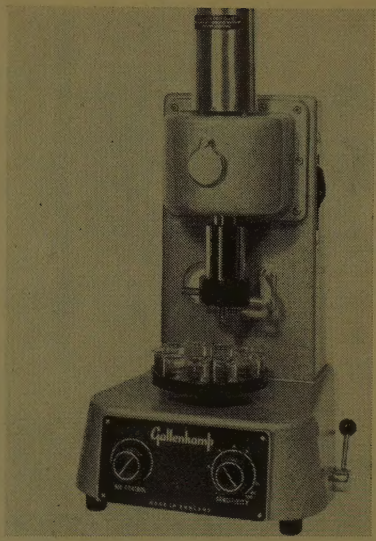

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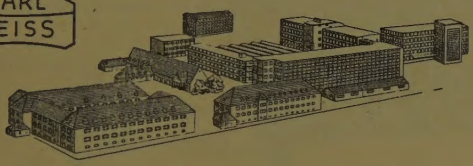

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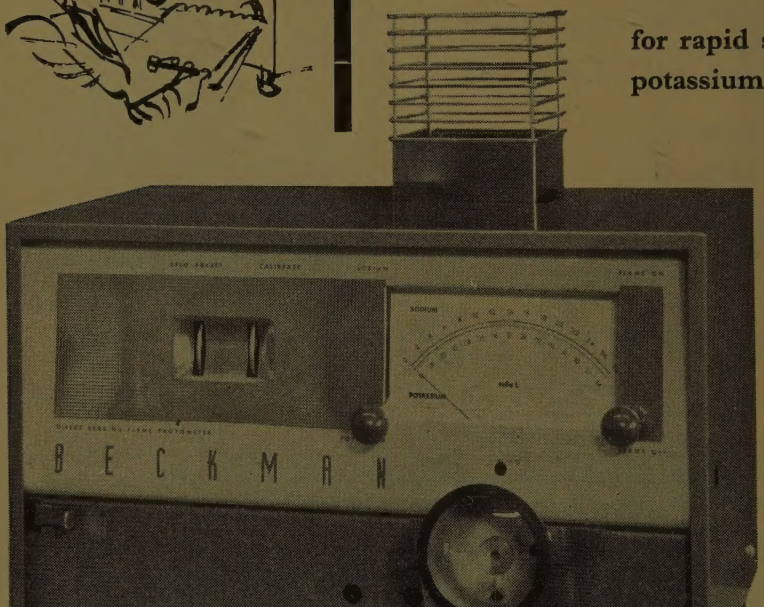

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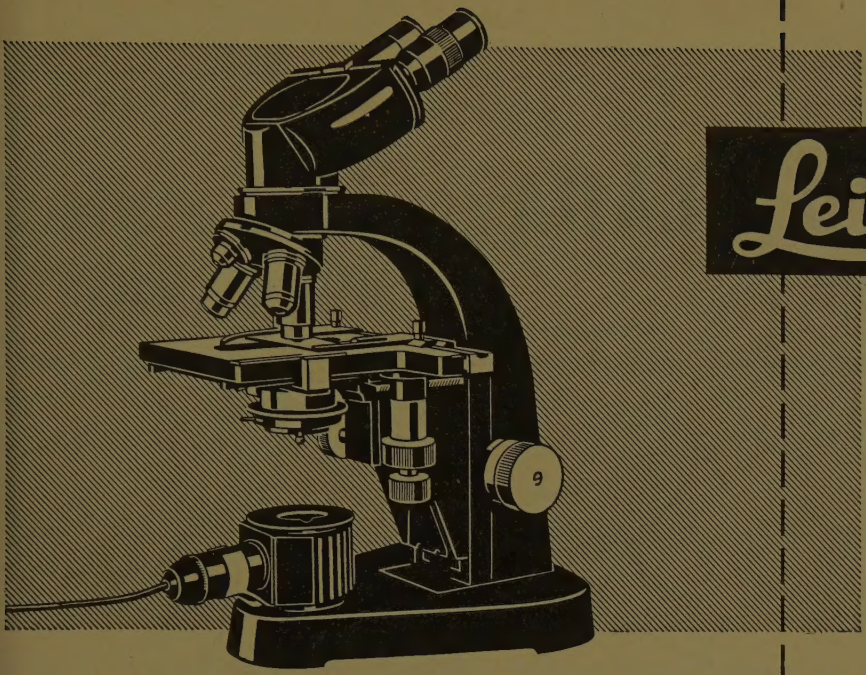

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SERUM LIPIDS AND LIPOPROTEINS IN OLD AGE[1]

By E. A. NIKKILÄ and T. NIEMI

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(Received for publication October 10, 1956)

All the circulating plasma lipids exist in form of water-soluble lipoproteins, which y be characterized by their flotation rate ultracentrifugation in solutions of suffi-ntly high densities, by their migration ocity in electrophoresis, and by their solu-ty properties in, *e. g.*, ethanol-water mix-es. All these methods can also be used the quantitative determination of indi-ual lipoproteins. Human as well as many mal sera have been demonstrated to con-n two major classes of lipoproteins called gh density" and "low density" lipopro-ns, or alpha and beta lipoproteins, respec-ely.

During recent years much evidence has n accumulating which indicates a relation-p between the concentration of beta lipo-teins and the development of atheroscle-is. One of the experimental findings, ch most strongly supports this concept, is the parallelism between the grade of coronary atheromatosis (as evaluated both clinically and anatomically) and the plasma level of beta lipoprotein in regard to age and sex in unselected or in apparently healthy populations (Jones, Gofman, Lindgren, Lyon, Graham, Strisower & Nichols, 1951; Barr, Russ & Eder, 1951; Nikkilä, 1953; Gofman, Glazier, Tamplin, Strisower & De Lalla, 1954; Tamplin, Strisower, De Lalla, Gofman & Glazier, 1954). In men the anatomical grade of coronary atherosclerosis has been shown to reach a maximum in the sixth decade of life, decreasing somewhat thereafter (*e. g.* White, Edwards & Dry, 1950), whereas in women the altheromatous lesions increase in severity up to the eighth decade, approximating first then the maximal level attained by men (Ackerman, Dry & Edwards, 1950).

In the present investigation the results of serum lipid and electrophoretic lipoprotein analyses of old persons 65 to 91 years of age are presented. The study was undertaken

[1] This work was aided by a grant from Societas ontologica Fennica.

to provide information additional to the data previously published by one of us (Nikkilä, 1953) on serum lipids and lipoproteins in healthy adults and, secondly, to determine the extent to which the sex difference in the increment of atherosclerosis at old age is paralleled by changes in the serum lipid and lipoprotein levels.[2]

MATERIAL AND METHODS

The material of this study was derived from 38 inmates of the Municipal Home of Helsinki. Hospitalization of these individuals was mainly for social reasons. Patients with an increased S. R. or with any known acute illnesses were excluded, whereas persons with an elevated systolic blood pressure, signs of myocardial degeneration (not uncompensated), pulmonary emphysema, *etc.*, were not excluded. None of the subjects had a verified myocardial infarction in their past history but some had survived cerebral vascular accidents of a minor degree. It is thought that the material represents an average sample of persons at these age levels. There were 18 women with a mean age of 77 years and 20 men with a mean age of 76 years.

All blood samples were drawn from the subjects in the morning after a fast of approximately 10 hours. The analyses were performed within 30 hours from the withdrawal and the sera were stored until this time at +4° C. The methods used were exactly the same as used in the previous study (Nikkilä, 1953). Thus the distribution of the lipids into the three main lipoprotein classes, alpha$_1$, alpha$_2$, and beta, was determined by fractionation of the serum by paper electrophoresis, with subsequent elution and chemical analysis of cholesterol and lipid phosphorus from the separated fractions. In our experience this procedure has been shown to be definitely superior in accuracy and reproducibility to the lipid staining methods.

[2] Some data of this investigation have been published in connection with two congress abstracts (Nikkilä, 1955 a, b).

Table I. *Serum cholesterol level and i distribution in three main lipo-proteins 38 individuals 65 to 91 years of age*

	Men	Women
Total cholesterol mg/100 ml	239 ± 16.0 125 — 422	298 ± 16 200 — 44
Cholesterol bound in alpha$_1$	44.5 ± 3.2 22.2 — 80.2	61.3 ± 4 25.0 —104
alpha$_2$	23.8 ± 2.4 9.2 — 53.3	25.9 ± 2 12.8 — 43
beta	167 ± 12.8 81 — 298	206 ± 12 138 — 30
Percentage of total cholesterol in alpha$_1$	19.5 ± 1.3 8.5 — 31.0	20.6 ± 1 12.5 — 27
alpha$_2$	9.9 ± 0.6 6.1 — 16.4	8.8 ± 0 5.3 — 15
beta	69.1 ± 1.3 57.9 — 81.7	68.9 ± 1 54.1 — 77

RESULTS

Serum cholesterol and its distribution in the lipoproteins.

These results are recorded in Table Among them the following should be emph sized. In old age, women appear to have considerable higher total serum choleste level than men, the difference in this ma rial being significant beyond the 1 per ce level. This difference becomes apparent the cholesterol bound to both alpha$_1$ and b lipoproteins, there thus being no sex diff ence in the percentage distribution of cho sterol in the lipoproteins. The sex differen in the absolute concentration of choleste bound to alpha$_1$ lipoprotein is significant the 0.2 per cent level, the correspondi value for beta lipoprotein being 3 per ce The alpha$_2$ lipoprotein-bound choleste shows no sex difference.

When compared to the data for younger e groups (Nikkilä, 1953) it is seen that ither total cholesterol nor any of the lipo- otein cholesterol values of *old men* differ gnificantly from those of men between 40 d 60 years of age. After the 70th year ere occurs, however, a considerable drop the cholesterol levels of men, as a result which the difference between age groups to 90 and 40 to 60 is highly significant. contrast to this situation in men, women tween 60 and 80 years have a higher total olesterol concentration than women be- een 40 and 60 years. At least in the pre- nt material no decrease occurs before the th year of life. In the percentage distribu- n of cholesterol in the lipoproteins there no significant difference between the age oups 40—60 and over 60 either in men in women.

Individuals with an elevated systolic blood essure had a somewhat higher total chole- erol and beta lipoprotein cholesterol than rmotensive subjects, but neither of these fferences was significant. On the other nd, a rough correlation was found be- een the roentgenologically evaluated calci- cation of the thoracic aorta and the total olesterol level (on an average 230 mg/100 in X-ray negative, 258 mg/100 ml in lcification of a slight degree and 281 mg/ 0 ml in gross calcification). If the degree aortic calcification is grossly graded from to 3, the average severity for men in the esent series was evaluated as 1.7 and for men as 2.4. The aortic calcification was us more advanced in women than in men.

Table II. *Serum phosphatide level and its distribution in three main lipo-proteins in 38 individuals 65 to 91 years of age*

	Men	Women
Total phospholipid mg/100 ml	254 ± 16.5 171 — 444	307 ± 13.7 222 — 424
Phospholipid bound in alpha$_1$	87 ± 8.2 47 — 130	109 ± 7.2 71 — 151
alpha$_2$	27 ± 4.7 15 — 36	25 ± 3.3 11 — 49
beta	131 ± 12.2 103 — 209	162 ± 10.2 117 — 212
Percentage of total phospholipid in alpha$_1$	34.0 ± 2.0 23.2 — 45.4	37.1 ± 2.2 29.1 — 48.2
alpha$_2$	11.3 ± 2.3 6.9 — 23.2	8.7 ± 1.4 3.2 — 20.3
beta	55.3 ± 2.2 46.9 — 62.4	54.4 ± 2.1 46.4 — 65.2

Serum P-lipid and its distribution in the lipoproteins and the cholesterol/P-lipid ratio.

The mean values, standard deviations of the means and the range are summarized in Table II. As regards the differences between the two sexes at old age and between the various age classes of the same sex, the statements made above for cholesterol are valid also for P-lipids. In a way this is also apparent from Table III, which contains mean values for the C/P ratio of whole serum and of the three lipoproteins in both sexes. No significant sex difference is manifest in any of the ratios. In this connection, again, the remarkable constancy of the C/P ratios of the human serum lipoproteins even when separated by different means is noteworthy. The mean values recorded for beta lipoprotein range from 1.24 to 1.35, and for alpha$_1$ lipoprotein from 0.39 to 0.63 (Russ, Eder & Barr, 1951; Kunkel & Slater, 1952;

Table III. *Cholesterol/phosphatide ratio of total serum and of the three main lipoproteins in 38 individuals 65 to 91 years of age.*

	Men	Women
Serum	0.94	1.00
$Alpha_1$	0.50	0.56
$Alpha_2$	0.88	1.04
Beta	1.27	1.27

Nikkilä, 1953; Hillyard *et al.*, 1955; Havel, Eder & Bragdon, 1955).

DISCUSSION

The most significant observation derived from this study is the marked sex difference in the serum cholesterol and phospholipid levels which manifests itself in old age and which is due to a sharp decline occurring in males after the 65th year with a simultaneous increase in females. Due to the high grade correlation between the total cholesterol concentration and the beta lipoprotein-bound cholesterol, this difference is reflected also in the beta lipoprotein cholesterol (and phospholipid) values. This result is in close agreement with those of Gertler & Oppenheimer (1953), whose study makes so far the most detailed contribution to the subject of serum lipids and lipoproteins in old age in both sexes. Also Glazier, Tamplin, Strisower, DeLalla, Gofman, Damber & Phillips (1954), in their extensive ultracentrifugal study on the concentration of the various lipoprotein classes in an unselected human population, noted that the rising trend with age continues in females up to the seventies, whereas the males reach a maximum at about the 60th year. From their data it is further apparent that after the 60th year the females have an equal or higher concentration of the various low density lipoproteins in their serum than the males. Unfortunately, records for age classes over 70 a lacking in this material.

In persons past eighty Eiber, Goldbloom Boyd, Chapman & Deutschberger (1954 have found a lower level of S_f 0—12 a 12—400 class lipoproteins but no differenc in the total cholesterol and phospholipi concentrations as compared to younger indi viduals, and no significant difference be tween the two sexes. In the material o Ackermann, Buehler, Toro & Kountz (1955 comprising males aged 53 to 94 years the S 12—20 level remained constant up to th 80th year and decreased thereafter. Thes results thus differ from those mentione above without its being possible to find an reasonable explanation for the divergenc

Any attempt to explain these age change of serum lipids is at present met with con siderable difficulties. The results of Key Fidanza, Scardi, Bergami, Keys & DiLorenz (1954) and of Keys, Vivanco, Miñon, Key & Mendoza (1954) on the serum cholestero among various nations suggest a dependenc of the age trend of serum cholesterol lev on the level of habitual fat intake befor middle-age but are hardly applicable t account for the observed changes in seru lipids in old age. Keys, Mickelsen, Mille Hayes & Todd (1950) have suggested tha the fall in the serum cholesterol level afte the age of 60 in males is not real but caused by a better survival of originall hypocholesteremic than hypercholesterem individuals. However, the mortality rate i diseases associated with hypercholesterem as compared to other causes of death seem not to be high enough to account for th effect entirely. In terms of the well-know

neral principle of the factors maintaining serum level of a given substance, a de- ase in the serum cholesterol is either due a decreased synthesis (or intake) or in- ased excretion, or to both. In rats, Rosen- n & Shibata (1952) have demonstrated decrease of the rate of cholesterol syn- sis with increasing age. In man the prob- n remains obscure until the accumulation further data on the regulatory mechanism cholesterol homeostasis and on the basic chemistry of aging.

A bulk of evidence now exists to indicate t estrogenic hormones tend to lower the um cholesterol level (*e. g.* Barr, 1953; iver & Boyd, 1954; Russ, Eder & Barr, 55) possibly by depressing the cholesterol nthesis in the liver (Rosenman, Friedman Byers, 1952). This could be taken as an planation for the rise in the serum chole- rol levels after cessation of the gonadal nction in women. However, the use of physiological doses of estrogens in the periments referred to and the fact that no nificant sex difference of serum lipid els exists at the fertile age strongly argues inst the acceptance of this concept.

Finally, some remarks seem warranted on correlation of atherosclerosis and serum ds in old age. A comparison of the auto- data (White, Edwards & Dry, 1950; kerman, Dry & Edwards, 1950) with um cholesterol levels in both sexes at age sses above 60 apparently speaks against concept of atherogenicity of hypercholes- emia. In spite of their high serum cho- erol and beta lipoprotein concentrations, men at this age have on an average still coronary atheromatosis than men with ch lower serum levels of these substances. This fact has led Gertler & Oppenheimer (1953) to deny the importance of the mere serum cholesterol level in the genesis of atheroma and to emphasize the value of the cholesterol/phosphatide ratio instead. However, in view of the accumulative character of atherosclerosis there is little argument to exclude the possibility that in men the development of atheromatosis has mostly taken place during the preceding decades with more atherogenic composition of plasma, while in women the *rate* of coronary *atherogenesis* increases first in the postmenopausal period and manifests itself as increased *atherosclerosis* not before one to two decades later. This concept is fully compatible with the autopsy findings and with experience of the incidence of clinical coronary disease (*e. g.* Oliver & Boyd, 1956). There is but one further aspect which necessarily should be considered in the evaluation of this problem. As demonstrated earlier by Epstein & Boas (1954) and Eiber *et al.* (1954) and supported also by the X-ray findings in the present material, the incidence of aortic atheromatosis (as reflected by aortic calcification) in persons past sixty is definitely greater in women than in men, resulting in an equally high incidence of total atherosclerosis in both sexes. There thus seems to be a close correlation between the serum lipids and aortic atheromatosis, whereas some protecting local factor in the female coronary arteries somewhat obscures this relationship in coronary atherosclerosis. Interesting enough, Pick, Stamler, Rodbard & Katz (1952) and Stamler, Pick, Katz, Lewis & Page (1952) have demonstrated that administration of estrogens to cholesterol-fed chicks inhibits the development of coronary

atheromatosis but has no influence on the lesions of the aorta.

SUMMARY

Serum cholesterol and phospholipid concentrations and their distribution in electrophoretically separated lipoprotein fractions were determined in 20 male and 18 female subjects 65 to 91 years of age. Women revealed significantly higher lipid levels than men. In women the serum lipids rise continuously with age up to at least the 80th year of life, whereas in men the rising trend ceases at about 65. The percentage distribution of the lipids in lipoproteins is identical in both sexes and accordingly the changes of lipid levels are reflected in both alpha$_1$ and beta lipoproteins.

The possible causes of these changes and their relation to atherosclerosis are discussed.

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A STUDY OF SWEAT ELECTROLYTE EXCRETION IN A PATIENT SUFFERING FROM CONGESTIVE HEART FAILUR

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Under normal circumstances the kidney regulates the body sodium content by balancing the output against the intake. In congestive heart diseases this regulation fails, the patient puts on weight and develops edema, and sodium nearly disappears from the urine. It has been maintained that not only the kidneys, but also other organs suppress the excretion of sodium in congestive heart failure. White, Gordon & Leiter (1950) and Berger & Steele (1952) demonstrated a lowered sodium concentration in saliva, and Berger & Steele further demonstrated suppression of sodium excretion by the colon of edematous patients, and a lowered sodium concentration in the sweat. In two patients accumulating edema fluid the sweat sodium concentration was 8 and 13 mEq/l respectively, against an average of 39 mEq in normal individuals. Berger & Steele maintain that the accumulation of edema is due to a suppressed excretion of sodium through the kidneys, the sweat and salivary glands, and the colon. In their opinion this retention is due to an excess of a desoxycorticosterone-like hormone, capable of suppressing the excretion of sodium through various organs. Robinson & Robinson (1954), however, seriously doubt the correctness of Berger & Steele's explanation and suggest that the lowered sodium excretion through the sk may be an adaptation to salt depletion caus by carboxylic resin therapy.

We have had the opportunity to study t excretion of electrolytes through the swe in a case of congestive heart failure. In th patient no peripheral edema appeared in spi of a very low renal sodium excretion. O study revealed that this was due to a ve heavy sweating which contained all the di tary sodium. The purpose of this paper to report the results of the study.

REPORT OF CASE

The patient, a 67 year old laborer, was admitt to the hospital because of dyspnea. He had nev been ill, but for the previous two years he h suffered from dyspnea on exertion; three mont before hospitalization he had to give up work'r One month before admission he was taken ill w sudden and pronounced substernal pains, radiati to the left shoulder and arm. This episode last for half an hour. An electrocardiogram show evidence of myocardial infarction. Gradually t dyspnea got worse, and he was confined to b He was admitted to the hospital because of ra aggravation of his condition.

The physical examination revealed a sever ill man. Respiratory movements were rapid a shallow. The temperature was normal, pulse r 104 per minute; the pulse was irregular. The s tolic blood pressure was 105 mm Hg and the astolic 70 mm Hg. There was peripheral cyano

no edema, and no venous congestion on the . The precordial dullness was enlarged, and the al impulse was felt 14 cm from the midline in fifth left interspace. An apical systolic murmur le 4 was heard. There was percutory dullness at right lung base and at auscultation impaired re- ation. No ascites was detected. The liver edge felt 1 cm below the costal margin. Urine: ific gravity 1023; a trace of protein was found, only on the first examination. Venous pressure 130 mm of water, circulation time (decholin) seconds. The electrocardiogram showed atrial illation and left ventricular preponderance.

X-ray study showed fluid in the right thoracic ty. The heart shadow was generally enlarged. pleural puncture gave 280 ml of clear yellow d. Renal function was normal both on admission later on, endogenous creatinine clearance being nal on several examinations.

He was put on a low sodium diet + digitalis. At t his condition improved, but still a certain ree of dyspnea and cyanosis continued. How- r, he never developed edema. Blood pressure a little below normal, on one occasion as low 90 mm Hg systolic and 60 mm Hg diastolic. dually his condition went downhill; death oc- red 5½ months after admission.

What especially troubled the patient was a very re sweating. Occasionally he was bathed in at, especially in the mornings. The body tem- ature was normal. The pleural effusion recurred, several pleural taps became necessary.

Our attention was directed towards the heavy piration and dyspnea, but also to the scanty esis, and to the fact that the patient, who ob- sly suffered from a high grade of congestive t failure, never developed peripheral edema. fluid intake was 1100 to 1400 ml a day, while ary output was 300 to 400 ml a day, occasionally 200 ml. As practically no sodium was found he urine, it was decided to study the electrolyte through kidneys, sweat and pleural taps. The that he was confined to bed made urine collec- very accurate. The diet was regular low so- n diet; by calculation it was found to contain verage of 26 mEq of sodium per day.

The electrolytes in the sweat were collected in following manner: A piece of gauze, 45 by 45 cm, was placed on the patient's chest and in the axillae during various periods of experimental conditions. After conclusion of each period the gauze was rinsed with distilled water which then was analyzed for sodium and potassium. At compution allowance was made for time and total surface to get a measure of the daily cutaneous electrolyte excretion. The daily urinary excretion of sodium and potassium was also determined; the electrolyte concentration in pleural taps was analyzed, and urinary and pleural taps volume measured. Plasma concentration of sodium, potassium, CO_2 content and chloride was determined twice a week.

Sodium and potassium were determined with a Baird flame photometer, lithium internal standard, chloride by the method of Schales & Schales, CO_2 in a Van Slyke manometric apparatus.

RESULTS

The total electrolyte loss was studied during a period when the patient was given a low sodium diet only, and in periods when he in addition was given urea, acetazoleamide, ammonium chloride and Carboresin. The low sodium diet alone was given for 59 days, each of the drugs for at least 10 days.

The results are summarized in the Table:

In the low sodium period the fluid intake was 1190 ml a day on an average, while the diuresis was 370 ml; consequently there was a deficit of 820 ml. In spite of this considerable deficit, lasting several weeks, no edema appeared, he did not put on weight, and pleural taps were not needed. There was, however, a very pronounced perspiration, and on examination the sweat revealed a sodium excretion of 26 mEq a day. The 24 hour renal sodium excretion was very low, averaging only 0.4 mEq, and it was concluded that practicallly all the dietary sodium passed out through the skin. The urinary excretion of potassium was 20 mEq per day, while the cutaneous excretion was

Table I. *Fluid intake, diuresis, sweat intensity and electrolyte excretion during the stud*

	Fluid intake (ml/day)	Diuresis (ml/day)	Pleural taps (ml/day)	Sweat intensity + + + + to +	Sodium excretion (mEq/day)			Potassium excretio (mEq/day)		
					Urine	Pleural taps	Sweat	Urine	Pleural taps	Sweat
Low sodium diet (26 mEq/day)	1190	370		+ + + +	0.4		26	20		32
Low sodium diet + urea 45 g/day	1200	630		+ + +	0.7		24	12		50
Low sodium diet + Diamox 250 mg every other day	1140	610	105	+ +	6.6	15	4.4	33	0.5	20
Low sodium diet + ammonium chloride 3 g/day	1160	660	190	+	0.5	26		29	0.9	
Low sodium diet + Carboresin 48 g/day	1385	920		(+)	0.3		3.6	4.8		17

32 mEq. Plasma electrolytes were normal, the sodium concentration a little above 140 mEq per liter, potassium 4.9—5.1 mEq, chloride a little below 100 mEq, total CO_2 content 30—34 mEq. The urinary pH was 5—6.

In the urea period diuresis increased to an average of 630 ml a day, while the fluid intake was practically unchanged. The diuresis deficit was consequently reduced to 570 ml on an average. The perspiration in this period was less severe. The urinary excretion of sodium was unchanged, the cutaneous excretion insignificantly lower than in the first period. Even in this period all loss of sodium was through the skin. The urinary excretion of potassium was a little less than in the first period, the cutaneous excretion 50 mEq. The plasma electrolytes were unchanged, the urinary pH 7—8. In neither of these periods were pleural taps neede X-ray study showed no increase of pleu effusion.

In the acetazoleamide ("Diamox") perio fluid intake was 1140 ml a day, diure 610 ml. Several pleural taps became nec sary, and on an average 105 ml of fluid w lost each day in this way. The sum of urina and pleural fluid volumes was 715 ml, a the fluid deficit less than before. The swe ing was reduced. The urinary sodium e cretion increased significantly, while t cutaneous excretion fell to 4.4 mEq a d Fifteen mEq of sodium were lost throu pleural taps. The total sodium excreti through kidneys, skin and pleural ta amounted to 26 mEq a day. The urina potassium excretion was a little higher th before, while the cutaneous excretion wa little less. The pleural fluid contained o

ignificant amounts of potassium. The sma electrolytes remained unchanged. e urinary pH went up from about 5 to -8.

In the ammonium chloride period, fluid ake was unchanged, and so was the uri- ry output. Pleural taps became necessary, l on an average 190 ml a day were lost s way. The fluid deficit averaged 310 ml day. The perspiration decreased a little. e urinary sodium excretion was very all; the pleural taps contained 26 mEq. o sweat test was performed in this period. e urinary potassium excretion was 29 mEq day; in the pleural taps 0.9 mEq. The sma electrolytes were unchanged with one ception: the potassium concentration in- ased to 6 mEq per liter. The urinary pH s low, about 5.

In the Carboresin period, fluid intake was newhat higher than before; diuresis in- ased to 920 ml a day. No pleural tap was quired. There was practically no sweating, l the patient stated spontaneously that he t much better than before. The urinary cretion of sodium was very low, and so s also the loss through the skin. The uri- ry excretion of potassium decreased to mEq, the cutaneous to 17.5 mEq. The sma CO_2 content decreased to 24 mEq liter, sodium to 131 mEq, and potassium 3.8 mEq. Carboresin medication was con- uently suspended. In this period the pH the urine increased from about 5 to 7—8.

DISCUSSION

There are, in a study like this, several rces of error. No attempt was made to asure total body water or plasma volume. attention was paid to fecal electrolyte excretion. This is, however, quite insignificant except in diarrhea. Nor was attention paid to insensible perspiration, which, however, does not concern electrolytes. Objections may also be raised against the collection of sweat. Because of identical results on repeated examinations some conclusions seem nevertheless justified.

Our patient was confined to bed, with a room temperature of about 20° C. He performed no work except that connected with eating, respirating and so on. The body temperature was normal. In spite of this he suffered from a very heavy sweating which may have amounted to more than 800 ml a day. The heavy sweating had no apparent effect except maybe the excretion of water which was not excreted through the kidneys. One may ask about the sequence: did the sweat intensity increase because of a scanty diuresis, or was the diuresis small because of the heavy sweating? This question is imposible to answer only on the basis of the figures in the table. They only tell us that the sweating was so heavy that in spite of a very low diuresis edema never appeared.

Water retention is, however, an insufficient explanation of edema, even if it has been postulated that retention of sodium is secondary to retention of water. The reason why our patient never developed edema is that he excreted all the dietary sodium through the skin. The sweat sodium concentration may be judged to about 30 mEq per liter (26 mEq/820 ml) on an average and is thus within normal limits. However, on considering this concentration of sodium in relation to the results obtained by Berger & Steele, the high sweat sodium concentration is conspicuous. Berger & Steele found,

as mentioned above, in two patients a sweat sodium concentration of 8 and 13 mEq per liter, respectively. In our patient the sweat sodium concentration may be judged to thrice this value. This shows that our patient was able to excrete a relatively concentrated sweat. The figures from the urea period show the same.

In the acetazoleamide period and in the ammonium chloride period, diuresis was higher than in the low-sodium period, but on a level with the diuresis in the urea period. On the other hand, pleural fluid accumulation took place, and pleural taps became necessary. The reason is not apparent. One might consider a common cause, *viz.* the acidosis. In favor of this explanation is the fact that the drug which provokes the most severe acidosis (ammonium chloride) was the most effective in reducing sweat secretion and reproducing pleural effusion. The mechanism is difficult to explain, and as this possibility was not considered at the time of our study, no trial was made with other drugs provoking acidosis.

Carboresin effectively reduced sweat intensity and increased diuresis. In this period the patient felt relatively well. Sodium disappeared almost completely from urine and sweat, and pleural taps were not required. This was due to the binding of the sodium in the intestine. The sweat sodium concentration in this period was low, and may have been about 8 mEq per liter, which agrees well with the figures of Berger & Steele. As our patient was shown to be able to excrete a more concentrated sweat, one must agree with Robinson & Robinson's conclusion: the low sweat sodium value in patients suffering from congestive heart failure and given carboxylic resin therapy is due to a adaption to salt depletion, not to the disease itself.

In regard to potassium intake and output we do not exactly know the potassium content of the diet. Our diets contain between 50 and 125 mEq of potassium per day. Most of it is excreted through the kidneys, 10—1 mEq through the feces (unless there is diarrhea); the excretion through the skin considered insignificant. In our patient the urinary potassium excretion on a low-sodium diet was 21 mEq a day; through the sweat was excreted 32 mEq. This was quite extraordinary. The fecal potassium excretion was not analyzed, but as the patient did not suffer from diarrhea, the fecal excretion may be estimated to only a few mEq a day. The total potassium excretion thus may be about 6 —70 mEq a day, which agrees well with available figures.

We were struck by the potassium loss through the sweat, both in the low-sodium period and in the other experimental periods. Even during the Carboresin period, when the urinary potassium excretion decreased to very low values (4.8 mEq a day), more than 17 mEq was excreted through the sweat. The sweat potassium concentration was not estimated directly, but most probably it was higher than normal (normal values are reported to 1 to 15 mEq per liter (Robinson & Robinson, 1954)). It is also obvious that the potassium concentration was higher than the sodium concentration; in every sweat examination more potassium was found than sodium.

Our case presented several electrolyte abnormalities, chiefly consisting in a high cutaneous and a low renal excretion of s

n, combined with a high cutaneous ex-
ion of potassium. The mechanism and
cause are unknown, but must in some
be related to the heart failure. A high
at potassium especially if combined with
omewhat lower sodium value, points to
ersecretion of aldosterone (Conn 1955).
necropsy offered no explanation. Ex-
t for myocardial hypertrophy and fibro-
no pathological changes were demon-
ted. The endocrine glands and the kid-
s were normal.

SUMMARY

A 67 years old man suffering from con-
tive heart failure developed no edema in
e of abolished urinary sodium excretion.
e dietary sodium was found to be excreted
ough heavy sweating. The cutaneous potassium excretion was distinctly higher than normal and exceeded the sodium excretion. The excretion of sodium and potassium under various experimental conditions is reported.

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STANDARD BICARBONATE, ITS CLINICAL SIGNIFICANCE AND A NEW METHOD FOR ITS DETERMINATION

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The acid-base status of the organism is uniquely determined by the following quantities, 1: the actual pH of the arterial blood, 2: the actual pCO_2 of the arterial blood, representing the respiratory component, and 3: the quantity that represents the non-respiratory factors (Astrup 1954, 1955). Generally, either the total CO_2-content or the CO_2-combining power of the serum is used for the last. None of these, however, is suitable because they will vary with changes of pCO_2 and oxygen saturation in the sample as shown in Table I. These changes may be considerable, even when the respiratory function of the patient is normal, *e. g.* when venous blood is used, when stasis is applied, or when the technique of blood sampling is not perfect anaerobic. All these errors can be avoided by determining the standard bicarbonate (Table I), which is defined as the concentration of bicarbonate ion in the plasma part of fully oxygenated blood at 38° C, when the pCO_2 is 40 mm Hg.[1]

From a clinical point of view, this quantity is an important one, as it is a reliable and probable the most convenient measure of the non-respiratory components of the acid

[1] In a previous work (1955) standard bicarbonate was defined with regard to the variation of the oxygen saturation of the sample. The new technique, described here, makes it more convenient to use the new definition.

Table I. *The content of total-CO_2, actual bicarbonate, CO_2- combining power and standard bicarbonate in plasma from the same blood sample at varying pCO_2 and varying oxygen saturation. — Expressed as mEq/l.*

	pCO_2 20 mm Hg	pCO_2 40 mm Hg	pCO_2 80 mm Hg	
Total-CO_2	16.8	22.2	30.0	
Actual bicarbonate	16.2	21.0	27.6	Fully oxygenated blood
CO_2-combining power	18.0	22.2	27.5	
Standard bicarbonate	21.0	21.0	21.0	
Total-CO_2	19.6	25.7	34.8	
Actual bicarbonate	19.0	24.5	32.4	Fully reduced blood
CO_2-combining power	21.0	25.7	31.9	
Standard bicarbonate	21.0	21.0	21.0	

e metabolism in the organism. As far we know, however, standard bicarbonate not yet been used in clinical work, owing he lack of a simple method for its deter-ıation (Van Slyke, 1932). In our labora-y a simple and rapid method for routine rk was developed, and standard bicar-ıate is now determined instead of total ^{2} for routine analyses.

n the following the method will be de-ıbed, and some relating and theoretical blems will be discussed. Finally, the ical use of the method will be commen-on.

METHOD

Principle

The blood is stabilized by heparin + sodium ride to prevent coagulation and glycolysis. In ater bath of 38° C the sample is equilibrated ı an O_2—CO_2-mixture of known composition ı a partial pressure of CO_2 near 40 mm Hg. gas mixture is bubbled through the blood to ch some silicon oil emulsion has been added prevent foaming and hemolysis. The pH of equilibrated sample is measured electrometri-y, and the standard bicarbonate can then be ved from the Henderson-Hasselbalch equation.

Reagents

Heparin-fluoride solution, containing 0.2 per cent heparin and 2.5 per cent of sodium fluoride R.) in water.

Silicon oil emulsion. "Antischaum-emulsion SE" n Wacker (Munich). This is an emulsion in cr with a high content of silicon.

O_2—CO_2-mixture. The mixture is commercially ilable in cylinders, ordered to contain 5.6 per of CO_2 and 94.4 per cent of O_2. The percen-e of CO_2 must be known exactly, *e. g.* from dane analysis.[1] The pCO_2 is calculated daily n the equation:

$$pCO_2 = \frac{B - 50}{100} \times CO_2\text{-per cent.}$$

B is the barometric pressure in mm Hg, 50 is the partial pressure of vapor at 38° C in mm Hg, and CO_2-per cent is the percentage of CO_2, v/v. The cylinder is placed in a horizontal position (Astrup, 1956).

Potassium chloride, A. R. As fine crystals, and as a saturated aqueous solution.

Standard buffer solution. Contains 1.8156 g KH_2PO_4 (Sørensen) and 9.4934 g Na_2HPO_4. $2H_2O$ (Sørensen) in 1000 ml of aqueous solution. The pH at 38° C is taken to be 7.360 according to Astrup (1956).

Apparatus

Glasses for collecting blood. Small test tubes, capacity 7—10 ml, with rubber stoppers. Each tube contains 1 mg of heparin and 12.5 mg of NaF, *i. e.* 0.5 ml. heat-dried of the solution given above. The temperature should not exceed 100° C during the evaporation, otherwise a partially insoluble "cake" will be formed.

Glasses for equilibrating blood. A convenient form and size is shown in Fig. 1. Each glass is provided with a rubber stopper with a long inlet tube and a short outlet tube.

Thermostat with gas distribution system. A glass vessel (42 × 28 × 28 cm) is used for the water bath. The temperature is kept at 38° C ± 0.1°.

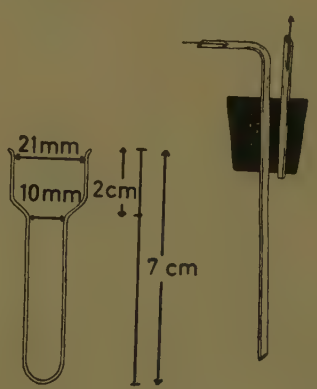


Fig. 1. Bubbling glass, and rubber stopper with inlet and outlet tube.

We are indebted to Cardiologisk Laboratorium, shospitalet, for analysing our gas mixtures.

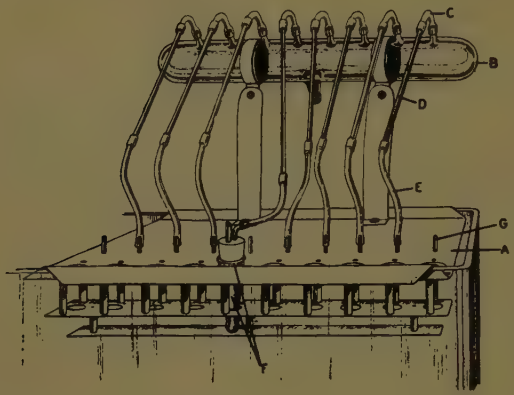


Fig. 2. Gas distribution system. The gas mixture is distributed through a glass comb (B), plastic tubes (C and E), and capillary tubes (D) to 8 bubbling glasses one of which is shown (F). The plastic tubes (E) are closed by short metal rods (G) when not in use. For dimensions, see text.

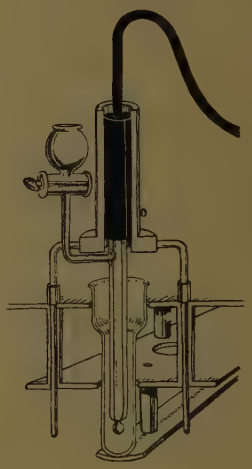


Fig. 3. Cross section of part of frame with bubbli glass. The electrode with electrode fork in positi for measuring pH.

A plate of stainless steel, Fig. 2, A, hangs on the edges of the glass vessel. The edges of the plate are bent in such a way that the plate itself is positioned 1½ cm below the edges of the vessel, *i. e.* just above the water level. Ten glasses for equilibration can be mounted in a row of holes (diameter 22 mm, center distance 30 mm) in the plate along one of its sides. A strip of stainless steel with smaller holes (diameter 12 mm) is held below the big holes by means of two distance pieces per hole. A strip of plate supports the glasses. The distance pieces are metal tubes. Each pair of them also serves as guiding tubes for the electrode fork (see below).

The gas mixture is fed to eight of the glasses for equilibration by the gas distributor, Fig. 2, B, (length 26 cm, diameter 4 cm) with one inlet tube and eight outlet tubes (outer diameter about 3 mm, center distance 30 mm). The inlet tube is connected to the gas cylinder by means of rubber tubes *via* an absorption flask with water, serving as a coarse flowmeter. Each outlet tube can be connected to a glass for equilibration by means of a plastic tube (6 cm), a capillary (length 15 cm, bore 0.5 mm), and a plastic tube (10 cm), Fig. 2, C, D and E. The capacity of the gas distributor in connection with the inserted capillari ensures a uniform bubbling rate in all glasses, ev with one or two plastic tubes freely open. Sho metal rods, Fig. 2, G, provide for closing the plas tubes when not in use.

The two test tubes for which gas supply is n foreseen are used for buffer solution and distill water.

Two long spirals of glass tubing are immers in the water bath (not shown). They provide f warming up the rinsing water for the electrode.

Electrode. The electrode used, Fig. 4, is man factured by Radiometer (Copenhagen), under t designation GK 2023. Important features of th electrode are the position of the calomel filling ne the glass bulb, ensuring the same temperature both components, and the use of a cotton plug a liquid juction. This plug has an advantage over fixed, porous plate, because it can easily be renew if abnormal diffusion potentials accur. It is renew by means of a fine pin. After replacement of t plug, the outer tube of the electrode should refilled with pure saturated potassium chlor solution by suction from the funnel, at the sa time removing air bubbles in the plug. Free c

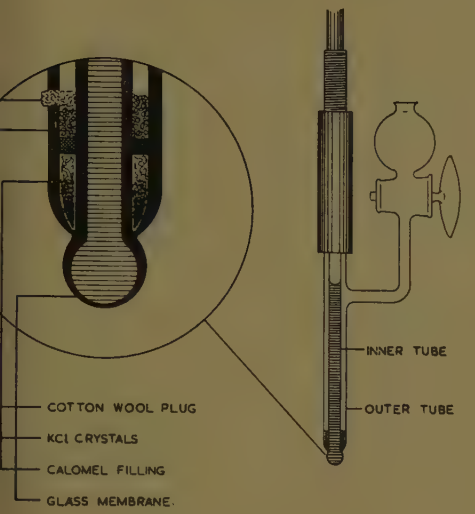


Fig. 4. Combined electrode, Radiometer type GK 2023.

, which are put down through the funnel, must ays be present above the calomel filling (Fig. 4). plug should be cautiously pressed so tightly that outflow of KCl-solution is just visible when stopcock is open. When not in use, the elec- e is stored in water in the thermostat with the cock closed.

he proportionality factor of the glass electrode ld be tested occasionally with a buffer solution a pH that differs sufficiently from 7.36. More ortant is the check of the diffusion potential in cotton plug. Here, anomalies may occur when suring on solutions containing proteins, parti- rly if the rinsing between measurements is fficient. Such abnormal potentials can be dis- red by comparing the "combined" calomel elec- e with an ordinary "free" calomel electrode r the same conditions (Astrup & Schröder,). The development of abnormal potentials also be discovered by measuring the pH of mple of pooled serum after equilibration with as for standard bicarbonate determinations. pool of serum is divided into small samples, h are stored deep-frozen. Each day one sample quilibrated with the gas mixture, and the pH easured. We make this check as a routine test, and have found it most useful.[1] Abnormal potentials are rare. The electrode can be used for a month or more without having the plug renewed.

Electrode fork. The electrode fork, Fig. 3, is made to receive the cap of the electrode, and the two legs (length 8 cm, diameter 5 mm, center distance 5 cm) will fit into any of the pairs of guiding tubes. The fork offers effective protection to the electrode during all manipulations.

pH-meter. The pH-meter must have an accuracy of at least ± 0.005 pH. The pH-meter, type PHM 3, made by Radiometer, is suitable. The accuracy of this instrumentt is ± 0.001 pH. When used in conjunction with the electrode type GK 2023, the actual pH-readings must be corrected by adding 5.000.

Procedure

Blood sampling. Approximately 5 ml of blood are shed from a vein into a test tube with heparin-fluoride. The test tube is stoppered and inverted. several times. The sample is now stable for several hours at room temperature.

Equilibrating. 3—4 ml of the stabilized, well mixed blood are transferred to a glass for equilibrating, and placed in the water bath. One drop of silicon oil emulsion is added. The glass is stoppered, and the gas mixture is allowed to bubble through the sample at a rate of 3—4 bubbles per second, *i. e.* 20—25 ml per min. When 30 minutes have passed the pH can be measured.

pH-measurements. The pH-meter is carefully adjusted by means of the standard buffer solution. The stopper with its tubes is removed from the sample, and the electrode is lowered into the blood, using the legs of the fork as a guide (Fig. 3). The stopcock of the electrode is kept closed. When the electrode potential has become constant, generally within 2 minutes, the pH is read.

---

[1] On the other hand if the electrode is known to be in order, and if corresponding values for pCO_2 and pH are known for the serum sample in question, the sample can be used to determine the pCO_2 in the gas mixture used for equilibration (Astrup, 1956). This fact can be utilized, when new cylinders ar taken in use.

Series analyses. While waiting for the electrode potential to become constant, the stopper with its tubes is disconnected and put aside. The sample, previously measured, is disharged, and replaced by a fresh sample, and the bubbling started. When the pH has been read, the electrode is thorougly rinsed outside the thermostat with running, distilled water, which has been tempered to 38° C by passing the spirals in the water bath. The stopcock is opened and closed again to renew the liquid junction. Excess water is removed with filter paper, and the electrode is immersed in the next sample.

During measurements, fresh standard buffer solution is always kept ready in the thermostat for frequent check of the meter. This buffer solution must be kept stoppered to prevent CO_2-absorption.

Calculation

If the pCO_2 of the gas mixture is exactly 40 mm Hg, the pH measured can be inserted in the Henderson-Hasselbalch equation,[1] and the standard bicarbonate is derived directly (Astrup 1956). It is recommended to tabulate related values of pH and standard bicarbonate.

If the pCO_2 employed deviates from 40 mm, the pH found must be corrected. This may be done by means of log pCO_2/pH graphs for blood with varying content of hemoglobin and base (*i. e.* varying standard bicarbonate). Such graphs are given below. However, when the pCO_2 used is close to 40 mm, *i. e.* from 36 to 44 mm, the difference in the slopes of the curves may be neglected, and the correction can readily be derived from the equation:

$$pH_{40} = pH_{measured} + 0.006 \times (pCO_{2,\ used} - 40)$$

Accuracy

To estimate the accuracy of the method, the errors have been divided in two groups. One group includes errors originating in the determinations of the gas mixture, electrode errors and meter errors. The other group comprises errors due to variations in hemoglobin content and degree of hemolysis of the samples. The errors of the first group have been evaluated by means of the daily measurements on the samples of pooled plasma during several months. The results of these measurements deviate ± 0.01 pH from the mean value. The errors of the second group, which are thoroughly discussed in the section "comments", are small, *i. e.* of the order of ± 0.005 pH. Thus it can be established that the accuracy of the determination of standard bicarbonate is better than ± 5 per cent.

COMMENTS ON THE METHOD

1. According to the definition, any method for the determination of standard bicarbonate must include two steps: equilibration of the blood at 38° C with an O_2—CO_2-mixture of adequate composition, and the final determination of the bicarbonate concentration in the plasma part. While working with a method based on measurements on completely hemolyzed blood, we found that gas can be passed through whole blood without causing appreciable hemolysis, if silicon emulsion is previously added. This finding enabled us to work out a simple technique for unhemolyzed blood without the use of ordinary tonometers, which are inconvenient in routine analyses when thermostating is required. For the determination of bicarbonate an electrometric technique is to be preferred, as it does not require separation of the cells from the plasma. The design of the type GK 2023 electrode, and the shape of the glasses for equilibrating provide for bringing the blood in contact with the electrode without disturbing the gas-liquid equilibrium. During the measurement the glass electrode is immersed 3—4 cm in the sample and the escape of CO_2 from the surface will not influence the gas-liquid equilibrium.

[1] The Henderson-Hasselbalch equation can be written: $pH_{40} = 6.11 + \log \frac{\text{standard bicarbonate}}{0.03 \times 40}$

. By experiments, the concentrations of arin and fluoride used were found to ilize the blood effectively without chang- the pH, and the concentrations are not ical in this respect, as they may vary 5 per cent. The silicon oil emulsion was found to have no influence on the pH. time required for equilibrating depends the rate of bubbling. At the above rate flow most samples are equilibrated in –20 minutes. Equilibrium will always ttained by fixing the time to 30 minutes.

. As mentioned above it is necessary to rect the pH found to that corresponding to $O_2 = 40$ mm Hg, if the pCO_2 in the gas ture deviates from this value. The cor- ion can always be accurately made, if exact slopes of the log pCO_2/pH graphs the blood samples are known. The varia- s in the positions and the slopes of the phs depend on the concentrations of noglobin and acid/base in the samples. To ify the simplicity of the method applied correcting the pH values, we made some estigations concerning the log pCO_2/pH phs.

Log pCO_2/pH graphs for blood with vary- concentration of hemoglobin. In a pre- us work (Astrup 1956) it is shown that log pCO_2/pH graphs for plasma are ight lines within the physiological varia- s of both pH and pCO_2. The same was nd for both plasma and whole blood by win, Gould, Nashat & Neil (1955). In investigations blood from the arm vein used, and stabilized as described above. t was centrifuged anaerobically, and blood ples with different concentrations of noglobin were made by adding or re-

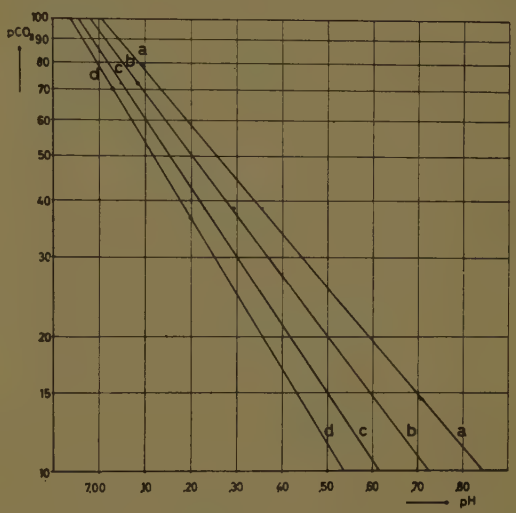


Fig. 5. Log pCO_2/pH graphs for hemolyzed blood with varying content of hemoglobin.
a. plasma
b. 7.3 g hemoglobin per 100 ml
c. 14.5 » »
d. 19.5 » »

moving plasma. The samples were hemolyzed by means of saponin and equilibrated with O_2—CO_2-mixtures of different pCO_2. The corresponding pH-values were measured as described for standard bicarbonate. As was to be expected, the log pCO_2/pH graphs showed increasing steepness with increasing concentrations of hemoglobin, as illustrated in Fig. 5. Similar results were obtained with blood from six normal persons. The graphs are displaced to the left as compared with graphs for unhemolyzed blood, because the erythrocytes are sligthly more acid than the plasma. We would emphasize that alterations in the slopes are small even with considerable variations in hemoglobin concentration. This means that when the pH is measured at a pCO_2 close to 40 mm, *i. e.* 36—44 mm, corrections to 40 mm can be

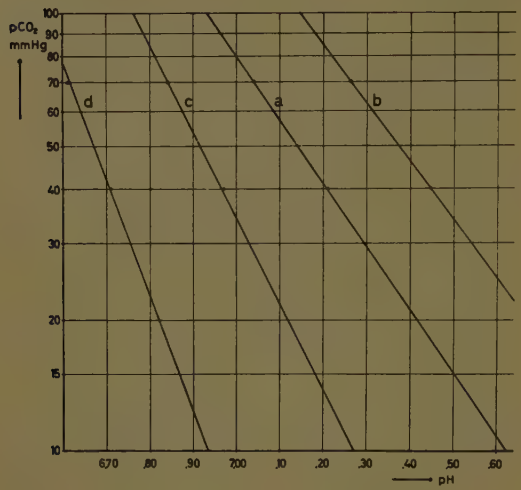


Fig. 6. Log pCO_2/pH graphs for hemolyzed blood with a concentration of hemoglobin of 15.1 g per 100 ml, after the addition of HCl or NaOH.

a. no addition
b. addition of 50 µl N NaOH per 2 ml of blood
c. » 25 » N HCl » »
d. » 50 » N HCl » »

made from a mean graph. The error introduced will be negligible, provided that the hemoglobin concentration is within 11 to 18 g per 100 ml.

Log pCO_2/pH graphs for blood with varying content of acid/base. Hemolyzed blood samples with varying contents of hemoglobin (about 0, 7.5, 15 and 19 g per 100 ml) were made as described above. In 4 ml of these samples pH was measured at different pCO_2 (20, 40 and 80 mm Hg). 25 µl of N HCl was then added to each sample, and after re-equilibrating with the pCO_2 mentioned, the new pH values were measured. This procedure was repeated with another addition of 25 µl of N HCl. Similar experiments were made with N NaOH instead of acid. In Fig. 6 are shown the log pCO_2/pH graphs for blood samples with hemoglobin concentrations of 15.1 g per 100 ml. As will be seen, the graphs are straight lines, the slopes of which increase with the amount of acid added. However, the differences in the slopes are small, and can be neglected in corrections for pCO_2 when this is close to 40 mm.

4. The slight hemolysis, which will often occur, can be neglected in routine work, which appears from the subsequent experiment. In 344 consecutive determinations of standard bicarbonate, the degree of hemolysis was estimated by cenrtrifugation of the samples after pH-measurements, and the hemoglobin content in the plasma was determined photoelectrically. The results are shown in Table II. In another series of experiments large samples of stabilized blood were equilibrated at 38° C with an O_2—CO_2 mixture of pCO_2 close to 40 mm Hg. The cells were separated from the plasma under the same conditions, and other samples with hemoglobin contents varying from 0 to about 19 g per 100 ml were made. These samples were hemolyzed by means of saponin, and

Table II. *The degree of hemolysis in 344 consecutive determinations of standard bicarbonate.*

Approximate content of hemoglobin in plasma in g per 100 ml	Number of samples
0	6
0.15	167
0.30	96
0.45	39
0.60	17
0.75	8
0.90	3
1.05	1
1.20	3
1.35	1
1.50	3

values were measured after re-equili-ing as for standard bicarbonate. In this it was found that the pH in the plasma eases about 0.010 for each g of hemo-in which has been liberated by hemo- in 100 ml of plasma. This shows that slight hemolysis that might occur during procedure described for standard bi-onate can be neglected. It was found by erience that the bubbling time should not ed one hour without checking the hemo- .

The procedure described has proved convenient and simple. It is less consuming than CO_2-analysis with the way or Van Slyke techniques. It should nentioned that the method can also be to advantage when determinations of O_2 in blood are made according to Astrup 56), as several samples of "separated" ma can then be equilibrated simultane-y.

APPLICATION OF THE METHOD IN CLINICAL WORK

he application of the method in clinical k necessitates the knowledge of normal es. Further, it is of interest to know relation between changes of standard rbonate and the amount of invading acid ase in the blood. Finally, it might be terest to compare the values of standard rbonate and of total CO_2 in the same ent.

Normal values. Normal values were rmined in the blood from 179 persons, nen and 85 women, aged 18—62, the ority being between 20 and 30 years old. mean value, M, was 21.7 mEq per l, the standard deviation, $\pm$ 1.4 mEq per l. Thus $M \pm 2\sigma = 18.9—24.5$ mEq per l. 80 per cent of the values were between 20.0 and 23.0 mEq per l.

The greater part (about 85 per cent) of the samples were from blood donors, from whom the blood was drawn at the end of the blood-letting. It is likely that some accumulation of lactic acid has occurred during the long period of stasis, so that the values tend to be too low. The time of the latest meal before sampling was not recorded. Many of the persons were no doubt in the phase of postprandial alkaline tide which will tend to give to high values. If blood samples were drawn from fasting persons with the usual degree of stasis, one would expect a narrower range of normal values than those found above.

The relation between standard bicarbonate and the invading amount of strong acid or base in the blood. This important problem was investigated as described on pp. 6 to 7 when obtaining the log pCO_2/pH graphs. Acid or base was added to hemolyzed blood with varying concentrations of hemoglobin, and the pH at $pCO_2 = 40$ mm was determined from the log pCO_2/pH graphs.

In Table III are shown the pH values of six samples with hemoglobin concentrations about 7.5 g per 100 ml, made from the blood of normal persons. Measurements on samples with hemoglobin concentrations of about 0, 15 and 19 g per 100 ml resulted in similar tables. By use of average values of pH, as will be seen in the last column of the table, the average concentrations of bicarbonate at $pCO_2 = 40$ mm were calculated. The value 6.11 was used for pK_1. When the concentrations of bicarbonate, thus calculated, are plotted against the amount of added

Table III. *pH values at pCO_2=40 mm in 6 samples of hemolyzed blood with concentratio of hemoglobin of about 7.5 g per 100 ml, before and after the addition of N HCl and N NaO*

Sample No.	1	2	3	4	5	6	Averag
Hemoglobin in g per 100 ml	7.1	7.3	7.5	7.8	8.0	8.0	7.6
25 mEq NaOH added per l of blood	7.51	7.47	7.51	7.51	—	—	7.51
12.5 mEq NaOH added per l of blood ..	7.40	7.41	—	—	—	—	7.41
No addition.....	7.27	7.26	7.28	7.28	7.27	7.26	7.27
12.5 mEq HCl added per l of blood	7.04	6.99	7.05	7.01	7.02	7.05	7.03
25 mEq HCl added per l of blood	6.71	6.66	6.65	6.69	6.72	6.72	6.69

strong acid or base, the graphs will be practically straight lines, as shown in Fig. 7. The slope of the lines indicates the buffer capacity of the samples. At each concentration of hemoglobin the difference between the values before and after the addition of acid (or base) will be a measure of the amount of acid (or base) added. At higher concentrations of hemoglobin a greater part of the acid or base added will be buffered by this protein, and the change in bicarbonate will be correspondingly smaller. At the concentrations of hemoglobin in our samples, *i. e.* about 0, 7.5, 15 and 19 g per 100 ml, the addition of strong acid or base will give rise to changes in the concentration of standard bicarbonate amounting to about 69, 56, 44 and 38 per cent respectively, expressed in percentages of the amounts of strong acid or base added.

In Fig. 8 is seen a graph relating the concentration of hemoglobin to changes of bicarbonate, expressed as percentages of strong acid or base added. The graph can be used to estimate the amount of invading acid or base in patients blood (calculated as strong acid or base). If, for instance, the hemoglobin concentration is 11 g per 100 ml, and the standard bicarbonate is 15 mEq per l, the blood has been invaded by an amount of acid which equals 13.4 mEq strong acid per l of blood. The deviation in standa bicarbonate from the normal value (21. is 6.7, and this will correspond to abo 50 per cent of invading strong acid.

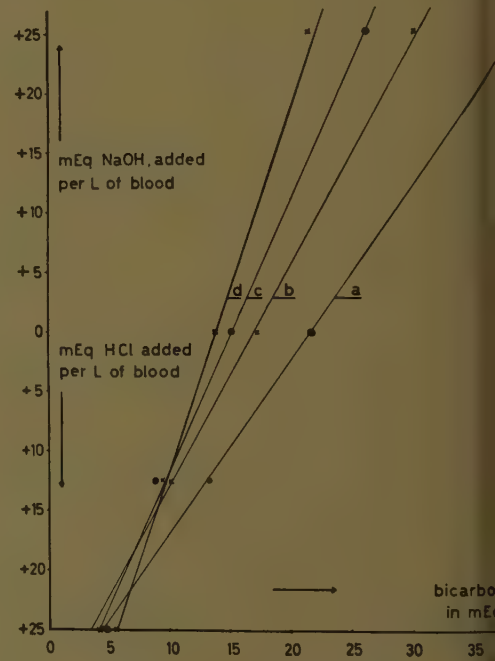
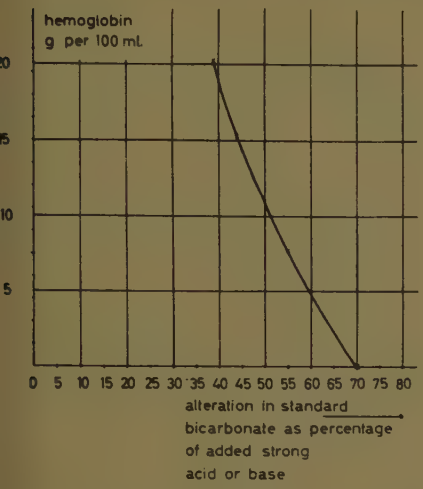


Fig. 7. Relation between added quantities of H or NaOH and values of bicarbonate at pC = 40 mm Hg in hemolyzed blood with differ content of hemoglobin.

a. plasma
b. 7.6 g hemoglobin per 100 ml
c. 15.1 » »
d. 19.0 » »


hemoglobin
g per 100 ml
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80
alteration in standard
bicarbonate as percentage
of added strong
acid or base

8. The change of standard bicarbonate, expres-
as percentages of strong acid or base added.
For further explanation, see text.

elation between standard bicarbonate and
-CO_2 in serum. In the case of 311 pati-
we supplemented the determination of
dard bicarbonate with determination of
l CO_2 in serum from blood drawn anae-
cally from the same venipuncture. The
-determinations were made with the
way technique, which has been used in
laboratory with a standard error of
.2 mEq per l (Jørgensen, 1956). In
9 are shown the corresponding values.
greater part of the values are within
normal limits. Low values of standard
rbonate correlate with low values of
-CO_2, as was to be expected. The
e holds true of many of the high
es of both quantities. 43 samples show
ormal total-CO_2 value, but either a too
(12 samples) or a too high (31 samples)
e of standard bicarbonate. 23 samples
v a normal standard bicarbonate, but a
high (18 samples) or a too low

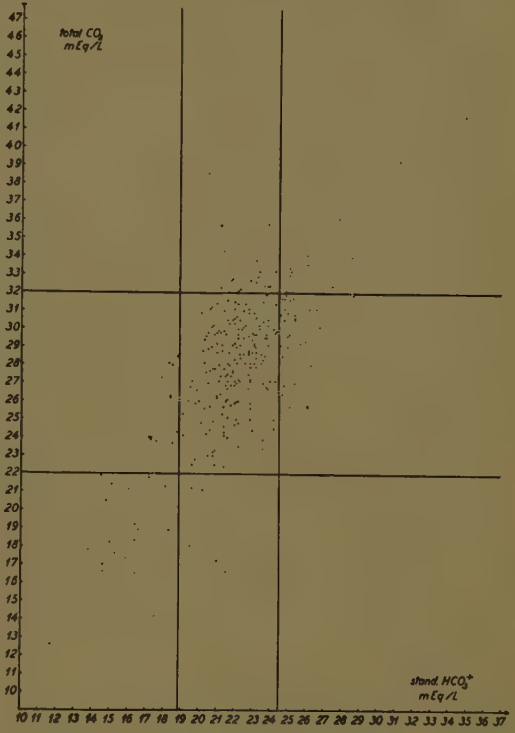


Fig. 9. Correlation between values of standard bicarbonate and of total-CO_2 in serum from 311 patients.

(5 samples) value of total-CO_2. Thus in 66 out of 311 cases the total-CO_2 value failed to give the information wanted about non-respiratory disturbances in the acid-base metabolism. The failures may be due to complicating respiratory disturbances or incorrect drawing of the blood.

SUMMARY

Standard bicarbonate, *i. e.* the plasma bicarbonate concentration in fully oxygenated blood at 38° C when pCO_2 is 40 mm Hg, is considered the best available measure of non-respiratory disturbances of the acid/base metabolism.

A method for determination of standard bicarbonate is presented and thoroughly discussed. The method requires about 5 ml of blood which can be taken without special precautions. The method is simple and has proved valuable in clinical routine work.

The relation between changes in standard bicarbonate and invading amounts of non-volatile acid/base in the blood is briefly discussed.

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URIC ACID, XANTHINE AND HYPOXANTHINE IN THE CEREBROSPINAL FLUID

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e concentrations of uric acid in the rospinal fluid, both the so-called normal ge and the normal range, are reported inconsistently in the literature. Thus rill (1931) found an average of 16.7 l in healthy persons when Folin's pro-e (1924) was used, whereas Buchanan, & Christman's enzymatic colorimetric od revealed an average of only 2.4 μg/ml e hands of Wolfson, Levine & Tinsley 7).

e reason for these discrepancies and for the enormous concentration ranges d (Fig. 1) is undoubtedly the lack of ficity and sensitivy of the non-enzymatic iques, which most often show high fig- and a wide scattering of the analytic ts.

unded on the principles for the deter- tion of purine compounds (Kalckar,), very accurate, sensitive, and con- nt methods for uric acid determination described in detail by Prætorius (1949) by Prætorius & Poulsen (1953). In Jørgensen & Poulsen published a tech- which includes the determination of ine plus hypoxanthine. These proce- are readily applicable to plasma, serum, rine analysis, and allow, by only slight fication, accurate measurements on small

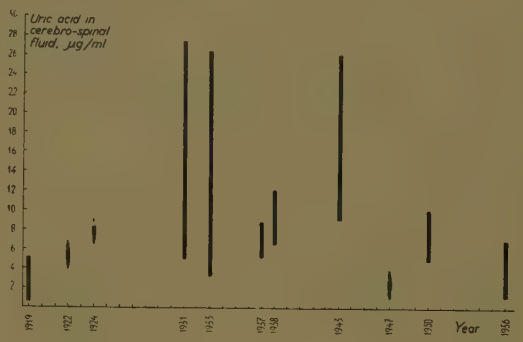

Fig. 1. Reports on the uric acid concentration in the literature through 35 years. 1956: the findings of the present authors.

amounts of cerebrospinal fluid, aqueous humor, tears, saliva, sweat, lymph, and joint fluid.

MATERIAL AND METHODS

Material

The determination of uric acid in the cerebrospinal fluid includes a total of 118 subjects, viz. 61 men and 57 women. The age distribution is recorded in Table II. A simultaneous analysis of the uric acid concentration in plasma and the cerebrospinal fluid was performed in 35 subjects. The concentration in the cerebrospinal fluid of xanthine + hypoxanthine was determined in 18 subjects, and in 22 post mortem cases.[1]

[1] We wish to thank the Director of the University Institute of Forensic Medicine, Prof. Knud Sand, M. D., for collaboration as regards the sampling.

The patients included were all suffering from surgical diseases, *e. g.*, hernia, ulcer, and fracture. No hereditary disposition for gout or other diseases of metabolism is on record, and no subjects with renal diseases are included in the material.

ANALYTICAL METHODS

Uric acid

Only slight modification made the technique (Prætorius & Poulsen, 1953) applicable in the analysis of cerebrospinal fluid. Because of the small content of protein it is not necessary to dilute this fluid as much as plasma. Hence uric acid can be determined with the same accuracy in both, despite the very low concentration of this compound in the cerebrospinal fluid.

The spectral change, which occurs upon the addition of glycine buffer with pH 9.3, represents a highly important source of error. Within the first 1—2 hours after buffering the fluid a pronounced fall in extinction will occur in the ultraviolet between 240 and 300 mμ; the decrease is maximal at 263 mμ and not at all negligible at 290—295 mμ, the maximum of the uric acid spectrum. The rate of the spectral change increases with pH and with temperature but not with the nature of the buffer as was previously suggested (Prætorius, 1949). Recently we showed that the change of the spectrum is due to an oxidation of ascorbic acid:

The spectral change from 240 to 300 mμ is quite similar to the spectrum of ascorbic acid. (Fig. 2). The addition of this compound to the buffered spinal fluid in the optic cell, when the spontaneous extinction fall has come to an end, produces an instantaneous rise of the extinction in the wavelength range, and this rise is proportional to the added amount of ascorbic acid. The rate at which the rise is abolished is quite similar to the rate of the spontaneous extinction fall, the cause of which, therefore, must be the original amount of ascorbic acid in the spinal fluid.

At 263—265 mμ the oxidation of 100 micromole ascorbic acid per liter corresponds to an extinction decrease of 0.110. When the cerebrospinal fluid is taken and kept under anaerobic conditions, there is

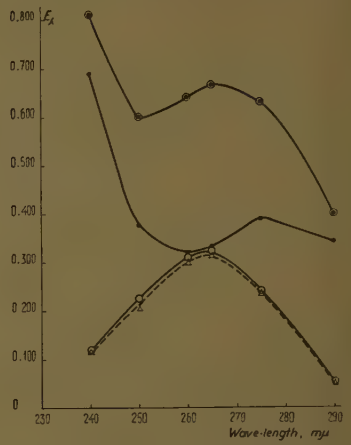

Fig. 2. Absorption spectrum of fresh cerebrospi fluid immediately after buffering at pH ⊙—⊙), and 3 hours later (●—●). The ference between these two spectra (o——o) is e to the spectrum of ascorbic acid (△—△, im diately after its addition to the buffer or to buffered spinal fluid. The added amount of asco acid made a concentration of 300 micromole per liter.

agreement between the measurements of asco acid determined by the dinitrophenylhydrazine thod (Daubenmerkl, 1949) and the spontane extinction decrease at 265 mμ.

Failure to oxidize all ascorbic acid of the sp fluid before the addition of uricase results in fal high figures of the uric acid concentration. room temperature the ascorbic acid in the unb fered cerebrospinal fluid is slowly oxidized; w buffered at room temperature (22°) the spec change is finished in 1—2 hours, and if the fered spinal fluid is heated in a water-bath to —90° the reaction is completed in 15 minu Recovery experiments showed that the uric content of the cerebrospinal fluid was unaffe by the procedures involving the oxidation of as bic acid, originally present or added to the f In our determinations of uric acid in plasma presence of ascorbic acid is not a source of er because the dilution ratio plasma/buffer is 1/4.

Technique

5 ml of cerebrospinal fluid is mixed with 1.5 ml M/15 glycine buffer of pH 9.3—9.4. The mixis heated for 15 minutes in a water-bath just he boil, cooled to room temperature, and placed e measuring cell. The reference cell contains uffer or only water. The enzymatic analysis rformed as in serum uric acid determination. ll analyses were run in duplicate although the rence between the two figures was always less 3 per cent.

Hypoxanthine and xanthine

hen the uric acid determination is finished the se in the measuring cell is destroyed by the ion of 200 μl 1.5 N—NaOH, which one minute is neutralized by 200 μl 1.5 N—HCl. pH is sted to 8.2 by 200 μl 0.6 M glycylglycine buffer 20 μl xanthine oxydase solution is added. (For preparation of this enzyme and the glycylcine er, see Kalckar, Kjeldgaard & Klenow, 1950, also Jørgensen & Poulsen, 1955). In less than hour the xanthine and hypoxanthine will be zed to uric acid. That this reaction is comd might be ascertained by checking that no ier increase of the extinction takes place at mμ. After changing pH to 9.3 by 300 μl 2M/3 ne buffer the formed uric acid is measured uricase against a reference cell with the same nts as the measuring cell.

he concentration of the two hydroxypurines expressed in terms of uric acid. The calculais based on the dilution ratio, which is 1/2.6, the fact that 1 μg uric acid per ml of the uring cell corresponds to an extinction de-e at 292.5 mμ of 0.0745.

ll hydroxypurine analyses were performed . Deviations from the mean never surpassed cent.

RESULTS

s in plasma and serum the average uric concentration in the cerebrospinal fluid igher in men than in women (Table I). distribution and uric acid concentrawithin 10-year groups are recorded in Table II. A moderate elevation of the uric acid concentration with age seems probable, but not definitely proved.

If normal range be interpreted as mean ± twice the standard deviation of the indi-

Table I. *The uric acid concentrations in cerebrospinal fluid from men and women. Mean, standard deviation of the individual observations, (s) and standard error of the mean (s.e.m.) expressed in μg/ml.*

Sex	Number of obs.	mean μg/ml	s μg/ml	s.e.m. μg/ml
M.	61	3.7	1.3	0.17
F.	57	2.7	1.0	0.14

Table II. *Age distribution of uric acid in cerebrospinal fluid after division of the observations into 10-year-groups.*

	Men		Women	
	Number of obs.	μg/ml	Number of obs.	μg/ml
21—30	2	0.21	4	0.27
31—40	5	0.22	9	0.22
41—50	16	0.33	15	0.25
51—60	9	0.38	12	0.23
61—70	21	0.41	10	0.34
71—80	7	0.43	3	0.41
81—90	2	0.32	1	0.47
91—100	0	—	1	0.50

Table III. *The uric acid concentration in cerebrospinal fluid from men and women. Mean and normal range in üg/ml. Number of observations within the normal range of variations (mean +2s).*

Sex	Mean μg/ml	Normal range μg/ml	Number of obs. within normal range
M.	3.7	1.1—6.3	59=97 per cent
F.	2.7	0.7—4.7	55=96 per cent

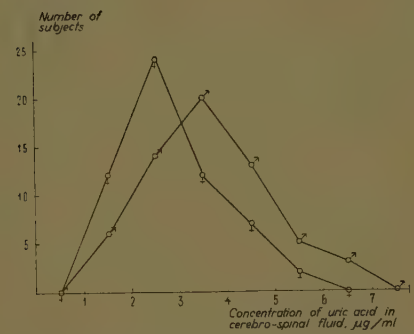

Fig. 3. The distribution of uric acid concentrations in cerebrospinal fluid from 61 men and 57 women. Class range: 1 µg/ml. Ordinates: number of subjects. Abscissae: concentration of uric acid in cerebrospinal fluid, µg/ml.

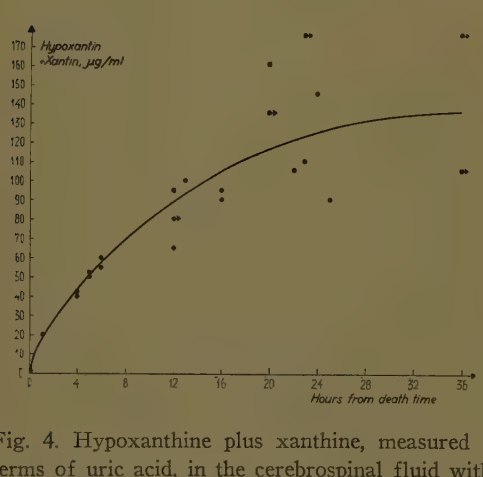

Fig. 4. Hypoxanthine plus xanthine, measured in terms of uric acid, in the cerebrospinal fluid withdrawn post mortem by puncture of *cisterna magna*. Abscissa: time after death, hours. Ordinates: concentration, µg/ml.

vidual observations, more than 95 per cent of the observations (Table III) will come within these limits, *viz.* in men 1.1 to 6.3 µg/ml, and in women 0.7 to 4.7 µg/ml. The few observations outside this range are all beyond the upper limit.

In 35 subjects, in whom the uric acid w measured simultaneously in the cerebrospi fluid and the blood plasma, the spinal flu concentration was found to average 6 per ce of the concentration in plasma; the dev ations ranged between 2 and 10 per cent.

It will be noted from Fig. 3 that the distr bution in men and in women is similar normal distribution curves.

The concentrations of hypoxanthine a xanthine were lower than the uric acid co centrations and averaged 0.97 µg/ml, t range being 0.25 to 1.50 µg/ml.

Fig. 4 shows a more than 100-fold increa of the concentration of hypoxanthine a xanthine in the cerebrospinal fluid aft death. These findings might be of intere in forensic medicine.

SUMMARY

The uric acid concentrations in the cer brospinal fluid from 61 men and 57 wom were analysed by enzymatic spectrophot metry. Mean ranges were 3.7 µg/ml a 2.7 µg/ml, respectively. More than 95 p cent of the observations ranged within 1.1 6.3 µg/ml in men, and within 0.7—4.7 µg/ in women. In a less comprehensive materi the concentration of hypoxanthine + xa thine was found to range between 0.25 1.50 µg/ml. After death this concentrati is increased 100-fold.

ACKNOWLEDGMENT

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ON THE DETERMINATION OF MAGNESIUM IN SERUM AND URINE BY THE TITAN YELLOW METHOD

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(Received for publication November 1, 1956)

The aim of this work is to investigate the applicability of the titan yellow method for the determination of magnesium, primarily in serum and urine.

The principle, first mentioned by Kolthoff (1927), is that titan yellow and magnesium hydroxide in an alkaline environment will combine to form a red lake, the intensity of which under adequate conditions will be proportional to the magnesium concentration.

The advantage of the method is, first and foremost, that it is quick and simple. A number of modifications adapted for various purposes have been described; for references to literature cf. among others Johnson (1952). The procedure used in this study is in particular based on works by Garner (1946) and Orange & Rhein (1951).

In what follows is given an account of the modification of the method we have arrived at as the one best serving the purpose, as well as a discussion of the various steps of the analysis.

PROCEDURE

Reagents: 1) trichloracetic acid 10 per cent
2) polyvinyl alcohol 0.2 per cent
3) titan yellow 15 mg per cent
4) sodium hydroxide 20 per cent
5) standard: magnesium sulphate solution with 2 and/or m.equiv. Mg/L.

Serum: 6 ml trichloracetic acid and 1 ml se are measured in centrifuge tubes, shaken, allo to stand for at least 10 minutes and centrifuga

2 ml of clear centrifugate is drawn by mean a pipette; 0.5 ml polyvinyl alcohol, 2.5 ml yellow and 1 ml sodium hydroxide are added. carefully, allow to stand for 10—20 minutes.

Standard: 1.0 ml magnesium sulfate solutio used instead of serum.

Blank: 1.0 ml distilled water is used instead serum.

Measurement: the extinction of the solutio determined by Beckman photometer, model wave length 540 mμ, 10 mm cuvette, reading against water.

Equation:

$$\frac{\text{ext. sample} - \text{ext. blank}}{\text{ext. stand.} - \text{ext. blank}} \times \text{conc. stand.} = \text{Mg m.e}$$

Urine: After careful mixing of the urine i treated like serum. Allowance to be made for color of the urine proper which is determined the extinction of a sample treated exactly as a lysis proper, but using 2.5 ml of distilled w instead of titan yellow. The extinction of the c proper is not determined as against pure water against a blank where the titan yellow is repl with water.

If the extinction of the urine exceeds 0.600 urine must be diluted.

Equation:

$$\frac{\text{ext. sample} - \text{ext. proper col.} - \text{ext. bl}}{\text{ext. stand.} - \text{ext. blank}} \times \text{conc. stand.} = \text{Mg m.eq./L.}$$

Duplicate determinations are used everywh

DETAILS OF METHOD

ıe principle as described by Kolthoff 7) is an adsorption of titan yellow on surfaces of colloid $Mg(OH)_2$ particles reby is produced a clear red lake and ı which is gradually separated a red ment.

ıe adsorption theory is confirmed by ıser & Dautzenberg (1952), who ex- ied the sediment by means of electron oscopy and X-ray diffraction. It thus to be made clear that it is a reaction of $OH)_2$ and not of free Mg ions.

s stabilizer to counteract sedimentation been used starch, gum ghatti and poly- l alcohol (Heagy, 1948).

ıe quantity of magnesium which theo- ally can be determined by this method estricted downwards by the solubility uct of $Mg(OH)_2$ (1.2×10^{-11}), up- ls partly by the quantity of colloid which be kept suspended by the stabilizer given rahamczik, 1949), partly by the quantity itan yellow which must be sufficient reaction wtih the whole quantity of $OH)_2$ in the sample. The procedure de- ed here is based on Mg concentrations 0×10^4 times above the lower limit.

range & Rhein (1951) have proved that protein bound Mg is determined by this od.

er's law. The practical application of method is conditioned by the fact that ntensity of the color is proportional to Mg concentration. This has been tested neans of known $MgSO_4$ solutions in led water, and the results are recorded e curve, Fig. 1(b). It will be seen that urve follows a straight course up to Mg entrations of about 10 m.eq./L. equal to an extinction of about 0.650. Here it deflects and in case of higher concentrations there is a slight fall even before the test shows macroscopically visible sedimentation consisting of coarse, red fluffs which sediment slowly leaving the supernatant fluid clear.

In spite of the fact that we have to do with a suspension of colloidal particles, and not with a solution, Beer's law is thus valid within the concentrations stated. Corresponding results have been reported by Garner (1946), Kunkel (1947), Craig (1951) and Orange & Rhein (1951). Glemser & Dautzenberg (1952) who use a quantity of titan yellow varying with the quantity of Mg (and by routine add $CaCl_2$) do not find the curve linear at the lowest Mg concentrations.

As it is of importance that the straight lined course of the curve is as long as practically possible, in particular as far as regards the urine examinations with the greatly varying Mg quantities, investigations on the importance of alteration in the various factors in that respect have been made.

Titan yellow (methylbenzothiazol-(1,3)-4,4′-diazoamminobenzol-2,2′-Na salt of disulfon acid) is a yellowish powder soluble in water. It is an acid-base indicator, the color of the solution being pure yellow below pH 12, changing *via* brown to orange at increasing pH. Abrahamczik (1949) states that it is only in an acid solution that titan yellow is in real solution while in alkaline environments it appears a colloid.

A solution with 50 mg/100 ml titan yellow in water is stable in a dark bottle for weeks and consequently this is usable as a stock solution while a 15 mg/100 ml solution has to be made afresh at few days' interval owing

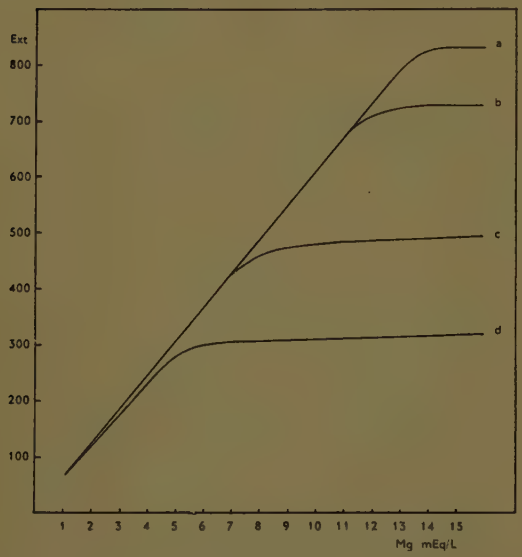


Fig. 1. The dependency of the extinction on the quantity of titan yellow. a) 20 mg 100 ml, b) 15 mg 100 ml, c) 10 mg 100 ml and d) 5 mg 100 ml.

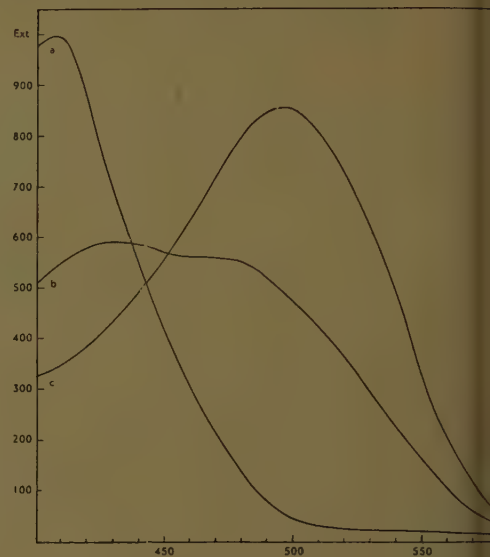


Fig. 2. The dependency of the absorption c on the individual components. a) titan yel b) titan yellow + trichloracetic acid + Na c) titan yellow + trichloracetic acid + NaOH polyvinyl alcohol in the proportion of quen used for the analysis.

to the fact that the extinction of the analysis becomes less the longer the solution of titan yellow has been left standing.

From Fig. 1 it appears that the slope of the curve is independent of the quantity of titan yellow to an essential degree, but that the deflection takes place at a smaller extinction value the less titan yellow one uses. Thus the quantity of titan yellow that is absorbed per unit $Mg(OH)_2$ is constant.

An absorption spectrum for a pure titan yellow solution of a concentration like that of the ready made test samples shows a pointed monophasic curve with a maximum about 405 mμ, Fig. 2(a) for the interval tested, 400—600 mμ.

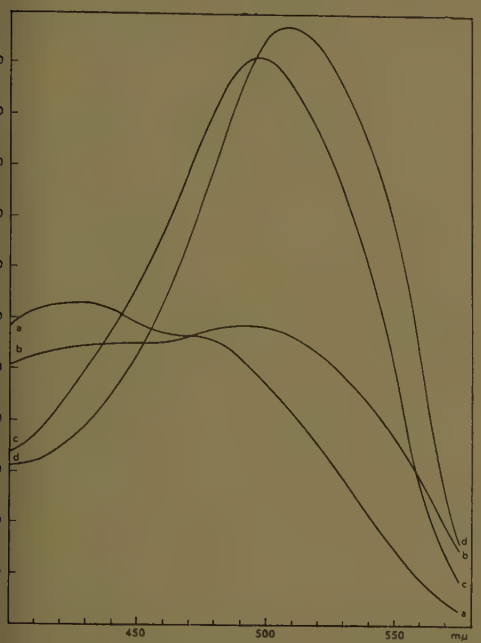
The other curves in Fig. 2 show the influence of the individual factors on the color of the solution as it appears at the analysis.

Trichloracetic acid reduces the intensity the color, but does not alter the princi course of the curve, and for this reason corresponding curve has been left out.

Sodium hydroxide addition gives the sorption curve (b) an essentially alte character with two flat maxima (about and 470 mμ).

Polyvinyl alcohol addition will produc new monophasic curve (c), now with maximum at about 495 mμ.

Fig. 3 illustrates the importance of polyvinyl alcohol addition partly to the sorption spectrum of the blank, (a, c), pa to the corresponding curve for a solu with 5 m.equiv. Mg per litre (b, d). F the curves it can be seen that the spectr



3. The dependency of the absorption curve on individual components. Trichloracetic acid con-ing solutions of a) titan yellow + NaOH, titan yellow + NaOH + $MgSO_4$ (5 m.eq. L.), c) titan yellow + NaOH + polyvinyl hol, d) titan yellow + NaOH + polyvinyl hol + $MgSO_4$ (5 m.eq. Mg/L.) in the pro-portion of quantities used for the analysis.

completely altered, that the polyvinyl al-ol increases the sensitivity (measured the extinction difference between the mple" and "blank" corresponding to mμ) by about 15 per cent, and that the ice of reading wave length is relatively levant in the range from 510—560 mμ if it is kept absolutely constant within each es of readings.

As the readings are made on a very steep t of the curve it follows that a known ndard must be included in each series ead of application of a fixed standard curve, cf. also the dependency of the ex-tinction on the age of the titan yellow solution.

Wave length. Repeated investigations on the importance of choice of wave length have shown that from 530—550 mμ the extinction curves are practically parallel on variations of the Mg concentration (cf. curves) and that thes ensitivity is most pronounced here. Maximum for these absorption curves is shown to change from about 500 to about 520 mμ by altering the Mg concentration from 0.5 to 10 m.eq./L.

It is also possible to determine the Mg concentrations by measuring the drop in the extinction occurring about 400 to 495 mμ with maximum at about 440, but the drop as expressed in per cent is smaller than the increase in the absorption at the wave length range chosen.

Influence of pH. By recording curves on the variations in absorption ratio with iden-tical Mg concentrations but with increasing OH^- concentration, the importance of the hydroxyl-ion concentration is shown to ap-pear as an increase of the color intensity.

However, it can be shown that the dif-ference of extinction with variations in the quantity of Mg decreases with incseasing pH, *i. e.* the sensitivity of the analysis abates. With about 3 per cent NaOH in the final solution, it has been found that the pH of the initial material does not essentially affect the result of reading, and at the same time one is working with a practically sufficient sen-sitivity.

Foreign ions. The importance of foreign ions has been treated by several authors, *e.g.* Glemser (1952) and Craig (1951), who em-phasize that a number of metal ions, in par-

ticular aluminium, copper, manganese, iron, calcium, and others may disturb the reaction either by forming colored hydroxides or inhibiting the formation of the titan yellow — $Mg(OH)_2$ complex.

Indeed, a consequence of this has been the development of a number of methods with addition of various salts to compensate this circumstance (*e. g.* Craig, 1951), while Abrahamczik (1949) prefers to separate these foreign ions.

In the examination of serum and urine most of these ions may, however, be left out of consideration as they occur in such small amounts — or in such constant proportions — that they are of no practical importance in this connection.

Investigations made by Garner (1946) and Orange & Rhein (1951) show that the calcium ion does not play any role under the test conditions applied.

Oxalate and phosphate have been investigated with a view to the influence of these ions on the color reaction by Orange & Rhein (1951), Garner (1946), and Heagy (1948) respectively, who find them of no importance.

All three ions have proved to be without importance to the accuracy of the analysis with the method described here.

Special studies on the influence of the Ca ion have been made by comparing the absorption curves (from 400—600 mμ) of solutions with and without Ca. It appears that up to 1 per cent $CaCl_2$ in the initial material does not affect the course of the curves in the interval between 510—560 mμ, while there is a slight reduction in the extinction in respect of solutions with Ca outside this interval.

The natural color of the sample is of i portance only in thec ase of urine where t procedure applied will generally give an e tinction of 0.010—0.020, but may rise about 0.050 corresponding to the extinct for a Mg solution with a concentration 0.8 m.eq./L. The value of the extinct of the natural color for this reason has t determined, yet it must be pointed out th the procedure reported here, *i. e.* determin tion against a blank, where the titan yello has been replaced with water, instead against pure water, will bring about an i creased accuracy of practical importan only in case of small Mg concentratio (at 1 m.eq. Mg/L. it may mean up to 5 p cent). Smaller values are but rarely fou in the urine.

The importance of the *time factor*: by r peated readings of the same sample it found that the color reaction appears pra tically momentarily and that within 3 hou hereafter an even and very slow drop in t extinction appears, parallel for the bla values and the Mg containing solutions. T drop, however, does not exceed 5 per cent these three hours.

If, therefore, it be decided to read betwe 10 and 20 minutes after the NaOH additi the time factor has no bearing on the res of the reading.

The *accuracy* of the method: In recove tests the method reported here has giv results which vary maximally $\mp$ 5 per c from what is calculated, while repeated te with the same samples of serum or ur give standard deviations between 2—3 cent in a series comprising 10 tests.

MAGNESIUM CONCENTRATIONS IN SERUM AND URINE

ormal values for serum magnesium have determined in 40 normal persons 20— ears of age. An average of 1.87 m.eq. L. (SD = 0.17) was found, correspond- to a normal range ($x \mp 2s$) between —2.21 m./eq./L.

his is in agreement with the results ob- d by a number of other authors by cation of titan yellow, Orange & Rhein 1), Kunkel (1948), and others.

rther, serum Mg has been determined 3 patients suffering from different dis-, those showing abnormal values of n K, Na, Cl, have been preferred, in- ng patients with uremia and shock. erally, values deviating significantly the normal stated were not found. ever, agonally values as high as up to m.eq./L. have been found.

he excretion of Mg in the urine per ours has been determined in a number patients on full ordinary diet. In 16 nts, where the function of the kidneys l be presumed to be normal and who not subject to treatment other than con- nent to bed, a total of 52 determinations Mg concentration in the urine were made. average excretion per 24 hours was nd to be 4.3 m.eq. with minimum at nd maximum at 8.3 m.eq. Mg.

rther investigations on the Mg content rine in the postoperative period are in ress.

SUMMARY

n account is given of the titan yellow od and of the influence of the analytic nts.

pecially it is shown that polyvinyl alcohol exerts a vigorous influence on the absorption spectrum and further increases the sensibility of the method.

Presence of oxalate, phosphate and Ca has no bearing on the result.

It is concluded that the method is well applicable to estimations of Mg in serum and urine.

ACKNOWLEDGMENT

I wish to thank Dr. T. Chr. Terkildsen, Chief of the Central Laboratory, for his never failing interest and help.

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Scandinav. J. Clin. & Lab. Investigation 144—149, 9, 1957.

POSITIVE WASSERMAN AND KAHN TESTS IN ENDOCARDITIS LENTA

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(Received for publication November 22, 1956)

Positive Wassermann and Kahn tests are not very unusual in endocarditis lenta patients. Most investigators believe, however, that endocarditis lenta has seldom resulted from syphilitic valvular defect. In the material of Landau & Held (1925), comprising 30 patients with endocarditis lenta, there were 10 cases with positive Wassermann reactions; the authors considered the reactions nonspecific. The 111 clinical cases of endocarditis lenta reported by Fulton & Levine (1932) included 11 instances of aortic valvular disease with a positive Wassermann test; only one of them could be attributed to syphilis. The present authors have previously (1956) reported on a material of 118 endocarditis lenta patients in which they found positive syphilitic seroreactions in 13 patients with aortic defects. Although some of them showed a history of syphilis, others had also had a rheumatic infection, and in the cases which were autopsied no evidence was seen to support the assumption that the endocarditis lenta had developed from a syphilitic defect of the valve. Moreover, Libman had already pointed out in 1917 how seldom endocarditis lenta was present when the valvular disease was of syphilitic origin. Rosenberg (1940), however, considered endocarditis lenta as a consequence of a syphilitic aortic valvu disease "no great rarity".

This is a study of the behavior of Wass mann complement-fixation (WR), choles rine Wasserman and Kahn flocculation te taken from 221 cases of endocarditis le treated in First and Second Medical Clin of Helsinki University, in the Medical D partements of Maria and Kivelä Hospi in Helsinki.[1]

TECHNIQUE OF WASSERMANN AN KAHN TESTS

The serological reactions were tested at Department of Serology and Bacteriology of H sinki University and at the State Serum Labo tory. The technique has been described in de earlier by Penttinen (1946). In some cases sitolipin test, V. D. R. L. flocculation test, v sitolipin as antigen (Uroma & Tuomioja, 19 was also made. The technical error of the Was mann, cholesterine Wassermann and Kahn t tions in Penttinen's (1946) material of 20 pregnant cases tested by the same laboratories 0.35 per cent. We can take it that the techn error for the present material is of the s

[1] We are indebted to the director of the I Medical Clinic Prof. William Kerppola, M. D., head of the Medical Department of the M Hospital Prof. Guido Tötterman, M. D., and head of the Medical Department of Kivelä Hos Prof. Pauli Soisalo, M. D., for making t material available for us.

r. In the present work, seroreaction + was idered strongly positive and marked + in the s below; + ?, ±, —? were considered weakly ive and all marked + ? in the tables I and elow.

CASES WITH POSITIVE SERO-REACTIONS

)f the 221 cases of the present material, showed positive or weakly positive ssermann, cholesterine Wassermann or nn reactions. In 11 cases the positive reaction was considered of possible hilitic origin, and in 50 cases the positive ctions obtained were considered non-cific.

The cases in which the positive seroreac- was considered definitely or possibly to syphilis are given in Table I. The e indicates the reaction as positive if any of the samples taken from the patient e a positive reaction. The past history nine of these patients included syphilis. the remaining two cases all the seroreactions of the patient were positive; in one (Case 6) they were checked twice and the reactions were compatibly positive, in the other (Case 7) the seroreactions were only made once. Seven cases showed a history of rheumatic infection; hence it is possible in these cases too that the endocarditis lenta had developed from a rheumatic heart lesion. Four patients showed failure of the aortic infection or congenital heart lesion, but in one of them only can endocarditis lenta be taken to have developed from a syphilitic aortic valvular disease. This patient (Case 11) suffered simultaneously from tabes dorsalis and syphilitic aortitis. Streptococcus viridans grew on blood cultures, and the patient died of bronchopneumonia after being given 80 million I. U. of penicillin and 20 g of streptomycin. Autopsy revealed syphilitic aortitis and changes of the aortic valve, plus soft vegetations in the endocardium below the aortic and mitral valves.

Table I. *The possibly specifically positive syphilis seroreactions of 11 endocarditis lenta patients.*

se	WR	Chol. WR	Kahn	Sito-lipin	History of syphilis	History of rheumatic fever	Remarks
	+	+	+	+	+	+	Cerebrospinal fluid WR+
	+	+	+	+	+	nil	Aortic valve defect
	+	+	+		+	+	Syphilitic mesaortitis
	+	+	+		+	+	Aortic valve defect
	+	+	+	+	+	+	No syphilis on autopsy
	+	+	+	+?	nil	+	Cerebrospinal fluid WR+
	+	+	+	+	nil	nil	No details available
	+	+	+	+	+	+	
	+	+	+	+	+	nil	Cerebrospinal fluid WR+. Syphilitic mesaortitis, mitral failure.
	+	+	+		+	+	No syphilis on autopsy
	—	—	+?	+	+	nil	Cerebrospinal fluid WR+. Syphilitic mesaortitis on autopsy.

Table II. *The nonspecific syphilis seroreactions of 50 endocarditis lenta patients.*

Case	WR	Chol. WR	Kahn	Sito-lipin	Remarks
12	+	+	+		Etiology of the heart disease obscure
13	+	—	—		History of rheumatic fever
14	+	+	+		No syphilis on autopsy
15	+	—	—		History of rheumatic fever
16	+	+	+	—	Etiology of the heart disease obscure
17	+	+	+	+	No syphilis on autopsy
18	+	+	+		—»—
19	+?	+	+		—»—
20	+?	+?	+?		History of rheumatic fever
21	+?	+	+		—»—
22	+?	+?	+?	—	—»—
23	+?	+?	+	+	No syphilis on autopsy
24	+?	+	+		—»—
25	+?	+?	—		Etiology of the heart disease obscure
26	+?	+?	+		—»—
27	+?	+	+		History of rhematic fever
28	+?	+	+?		No syphilis on autopsy
29	—	+	+		—»—
30	—	+	+	—	History of rheumatic fever
31	—	+	+	—	—»—
32	—	+	+	—	Congenital heart disease
33	—	+	+	+	No syphilis on autopsy
34	—	+?	+	—	History of rheumatic fever
35	—	+?	—		No syphilis on autopsy
36	—	+?	+?		—»—
37	—	+?	+		—»—
38	—	+?	+?	+	—»—
39	—	+?	+	—	—»—
40	—	+?	+		History of rheumatic fever
41	—	+?	+	—	Etiology of the heart disease obscure
42	—	—	+		History of rheumatic fever
43	—	—	+?		Etiology of the heart disease obscure
44	—	—	+	—	No syphilis on autopsy
45	—	—	+	+?	Congenital heart disease
46	—	—	+	—	No syphilis on autopsy
47	—	—	+	+?	—»—
48	—	—	+	—	—»—
49	—	—	+?	—	Etiology of the heart disease obscure
50	—	—	+		No syphilis on autopsy
51	—	—	+		—»—
52	—	—	+		History of rheumatic fever
53	—	—	+?	—	—»—
54	—	—	+?	—	—»—
55	—	—	+?	—	—»—
56	—	—	+?		—»—
57	—	—	+?		—»—
58	—	—	+?	+?	No syphilis on autopsy
59	—	—	+?		—»—
60	—	—	+?		History of rheumatic fever
61	—	—	—	+?	No syphilis on autopsy

Table II lists the seroreactions that were nsidered nonspecific in 50 endocarditis nta patients. The reaction is indicated as sitive if any one sample gave a positive sult, and as negative if the finding was gative for all the samples. For instance, e result WR+, Chol.WR+, Kahn+ does t necessarily mean that the tests with one d the same samples were positive; the sult may well have been composed of the ndings for several different samples.

The valvular lesion of the 50 endocarditis nta patients with positive syphilis reactions, ted in Table II, was not considered to be syphilitic origin for the following reasons:) they showed no past history of syphilis,) they showed a past history of rheumatic ver (18 cases), or nothing indicative of philis was found on autopsy (23 cases); o cases had a congenital heart disease,) when the etiology of the valvular disease as obscure a syphilitic etiology was often ntradicted by the patient's youth, or (4) e fact that no aortic lesion was found, (5) e positiveness of the seroreactions of the fferent samples showed considerable labi- y, one sample perhaps giving a single sitive reaction and then a negative reac- n at the control test; in addition, the dif- rent simultaneous seroreactions were often ntradictory (see Table III). For instance, a weakly positive Kahn reaction was ob- ined it was regularly checked, and the sero- actions of each patient were taken an erage of 3—4 times.

Table II shows that the reaction respon- ble for nonspecific positiveness was mostly ahn, which was positive in 90 per cent of e 50 cases considered as nonspecifically sitive. However although the Kahn reac-

Table III. *The results of the original sero-reactions of 7 endocarditis lenta patients with an unknown etiology of the heart lesion.*

Case	WR	Chol. WR	Kahn	Sito-lipin
12	—	—	—	
	—	—	+?	
	+	+?	+?	
	—	+?	—?	
	—	—	—?	
16	+	+	+	—
	—	—	+?	—
	—	—	—	—
	—	—	—	—
25	—	—	—	
	—	—	—	
	—?	—?	—	
	—	—	—	
26	—	+?	+	
	—	+	+	
	±	+?	+?	
	—	—	+	
	—	—	—	
41	—	—?	+	—
	—	—	+	—
	—	—	+	—
	—	—	—	—
43	—	—	—?	—
	—	—	—	—
49	—	—	+	—
	—	—	—	—

tion was positive in 89 samples from these 45 patients, it was negative in 71, 44.4 per cent, of the samples taken from the same patients. Table II shows further that the cholesterine Wassermann of 28 patients and the Wassermann of 17 patients were positive. A total of 74 samples was taken from these 17 patients, and WR was negative in 48 of them. The sitolipin reaction also was tested for 23 of these 50 patients; the result was positive in 8 cases.

Table III gives the original seroreactions of the 7 cases in which the etiology of the heart lesion was obscure.

For the 160 patients with negative syphilitic seroreactions the result was mostly based on a single negative sample. Had several samples been taken in these cases it is possible that they too might have included positive seroreactions.

Computed from the 221 cases of the total material, a non-specific positive WR was obtained in 17 cases or 7.7 per cent, a nonspecific positive cholesterine WR in 28 cases or 12.7 per cent, and a nonspecific Kahn reaction in 45 cases or 20.4 per cent, and nonspecific findings independent of the reaction were recorded in a total of 50 cases or 22.6 per cent.

DISCUSSION

The incidence of positive Wassermann and Kahn tests in the present material is fairly high. Among 61 patients with positive seroreactions there were 11 cases in which the positive seroreactions may have been due to syphilis, although endocarditis lenta had only developed from a syphilitic valvular defect in a single case. This amounts, for the material, to a rate of 5 per cent for positive syphilis seroreactions that are to be considered syphilis-induced, a rate which is somewhat higher than the lues incidence of these hospitals, 2.68 per cent, or 2239 instances of syphilis out of a total of 83 036 cases. These 2239 patients had tertiary syphilis, including cases in which the diagnosis of syphilis was made on the basis of medical history alone without any clinical or serologic indications of syphilis (lues medicata), and those in which only the positive seroreactions suggested the presence of syphilis. The latter cases, therefore, includ some of the cases considered as nonspecif in the present material. Hence it is possib that the positive seroreactions were now no specific in some of the syphilitic cases of th group now considered as syphilitic (11 pat ents). In an earlier material, published b Maijala, on seropositive tertiary syphil treated by Helsinki University Outpatie Medical Clinic in 1936—37, 10 954 cas included 3.44 per cent of syphilis.

The balance of the positive syphilitic ser reactions of the 50 patients must be co sidered nonspecific. The technical error b the same laboratories in a material of ov 20 000 patients was 0.35 per cent, negligib for the present study.

All this notwithstanding, it might see desirable to consider that the positive syph litic seroreactions taken as nonspecific we in fact due to syphilis, and that the labili or possible weakening of the reactions r sulted from the large doses of antibioti used to treat the endocarditis lenta. T elucidate this point, we have collected fro among the patients with tertiary syphil treated at our clinic (the Second Medi clinic) 14 cases treated with more than million I. U. of penicillin, and studied h the treatment given affected the positivene of the seroreactions. In four of the cas the positiveness of seroreactions was wea ened, or had even turned negative; this, ho ver, did not happen during the course treatment in the hospital but gradually ½—2 years. In the balance of 10 cases t seroreactions during this period continu strongly positive. This, we find, contradi the assumption that the syphilitic serore tions of the 50 endocarditis lenta patie considered nonspecifically positive mig

e been specific and the lability caused by tment.

s nonspecific seroreactions were found 2.6 per cent of the 221 endocarditis lenta ents of our material, these reactions are o great importance in the syphilis diag- s of endocarditis lenta patients. On the r hand, they may be of importance in irming the diagnosis of endocarditis a now that the classical picture of the ase has changed due to antibiotic treat- t, and diagnosis is complicated. Although onspecific positive seroreaction of this e is not comparable in diagnostic value the cardinal symptoms of endocarditis a, it is surprising that its incidence may al that of clubbing which was present in per cent of the 200 endocarditis lenta ents included in the material of Parsons, per & Scheifley (1953). But in severer s clubbing is more frequent; *e. g.* Kai- nen & Seppälä (1956) report an incidence 3 per cent in 118 cases.

lthough medical history data on syphilis always unreliable — "every syphilitic is ar" — it should be remembered on the s of both earlier investigations and the sent that the physicians in charge of ocarditis lenta patients should not un- essarily add the unpleasant diagnosis of hilis to the patient's list of diseases when diagnosis is based mainly on an erroneous luation of a nonspecific syphilis reaction. only gives the patient and his family ous psychic traumata.

SUMMARY

A positive or weakly positive syphilitic reaction (WR, Chol. WR, Kahn) was ed in 61 cases of a material of 221 endo- litis lenta patients. In 11 cases (5 per cent) the positive seroreaction was possibly due to syphilis; in only one of these cases was endocarditis lenta found to have developed from a proved syphilic valvular defect.

In 50 cases (22.6 per cent) the syphilitic reactions were considered nonspecific ; in addition to negative history of syphilis, the opinion was based on the autopsy finding, the great lability and mutual contradiction of the positiveness of the seroreactions, and the syphilis incidence of these hospitals which was 2.68 per cent of a material of 83 036 patients.

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THE EFFECT OF PHENTOLAMINE (REGITINE) ON THE PULMONARY CIRCULATION

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In a previous study on the effect of the ganglionic blocking agent hexamethonium bromide on the pulmonary circulation (Storstein & Tveten, 1954) we found that this agent had no dilating effect on the pulmonary blood vessels. The fall in pulmonary artery and pulmonary capillary venous pressure, which we observed, was interpreted as being due to a passive effect on the pulmonary circulation from the fall in systemic blood pressure and pooling of blood in the systemic circulation.

In the present study we intend to investigate the effect of the adrenergic blocking agent phentolamine (regitine) on pressures and flow in the pulmonary circulation.

We have been unable to find any previous study on the effect of this agent on the pulmonary circulation. But Dresdale, Michtom & Schultz (1954) using priscoline, which is pharmacologically related to phentolamine, found a distinct fall in pulmonary artery pressure combined with an increase in cardiac output in one patient with primary pulmonary hypertension and in another with pulmonary emphysema. They observed a greater fall in pulmonary than in systemic vascular resistance, and concluded that priscoline had a dilating effect on the pulmonary arterioles. On the other hand Mackinnon, Vickers & Wade (1956) in 4 patients with mitral pulmonary heart disease found no consist change in cardiac output, pulmonary art pressure or pulmonary resistance follow priscoline. In all cases they observed increase in heart rate, and in one case th was a severe rise in pulmonary artery pr sure combined with a fall in cardiac outp They did not think that the pressure in the pulmonary artery could be wh explained by the tachycardia, as the forn outlasted the latter. They supposed t priscoline may have a direct effect on arte smooth muscle, in the same way as anot adrenergic blocking agent, ergotamine(Go man & Gilman 1955).

MATERIAL AND METHODS

The material consisted of 16 patients with v ous types of heart disease (Table I). Three pati had normal pulmonary artery pressure, while other patients had pulmonary arterial hyperten up to as much as 73 mm Hg mean pressure.

The dose of phentolamine, which was to given during the investigation, was ascertained day before the catheterisation procedure, as dose necessary to produce a distinct fal systematic blood pressure.

The catheterisation procedure was carried as earlier described (Storstein & Tveten, 19 Owing to the rapid action of phentolamine (siding in 5—10 minutes) it was technically diff to make repeated determinations of cardiac ou

Table I. *Characteristics of the material for study.*

Name	Sex	Age	Diagnosis	Height cm	Weight kg	Body surface area m^2	Dose of pentholamine
E.H.	M.	53	Mitral stenosis	168	57.5	1.65	3
E.N.	M.	40	Mitral stenosis	173	68	1.82	5
J.N.	F.	60	Mitral stenosis, aortic stenosis & insuff.	168	53.2	1.60	5
A.K.	F.	60	Mitral stenosis	155	62	1.60	7
B.P.	M.	23	Pulmonary hypertension	186	67.5	1.90	5
H.H.	M.	48	Mitral stenosis	172.5	72.5	1.87	8
O.B.	M.	39	Ventricular septal defect, pulmonary stenosis	168	57	1.63	6
J.L.	M.	44	Aortic stenosis & insuff.	165	64	1.72	7.5
I.R.	F.	25	Mitral stenosis	158.5	60.5	1.62	7
O.D.	M.	22	Mitral stenosis, aortic stenosis & insuff.	172.5	66	1.78	6
P.P.	M.	51	Mitral stenosis, aortic stenosis & insuff.	172	62.4	1.75	5
B.M.	F.	34	Mitral stenosis	172.5	61.7	1.72	6
H.J.	M.	57	Mitral stenosis	173	58.8	1.70	5
B.H.	F.	51	Mitral stenosis	164	64.9	1.73	5
P.S.	M.	58	Silicosis	173.5	69.4	1.83	5
E.N.	M.	63	Mitral stenosis	167	64	1.73	5

V-pressure and pulmonary arteriolar resistance. only succeeded in doing this in 4 patients. The hial artery pressure was registered each minute during the first 5 minutes, and each rte during the next 5 minutes after injection ne drug. Mean pulmonary artery pressure was stered each minute, undamped pressure after d 10 minutes. Heart frequency was registered rocardiographically each minute. Mean brachial ry pressure was taken as diastolic + ⅓ pulse sure.

he statistical calculations have been carried by Mr. Per Johannesen, M. A. In the tables notes the observed value before phentolamine » » » after »

notes the difference between x and y ($z = x - y$)

$\bar{x}$, $\bar{y}$, $\bar{z}$ denote the arithmetic means of x, y and z respectively.

notes the sample standard deviation of z.

he effect of phentolamine has been tested by is of "Student"s two-tailed t-test (Cramér,), where $t = \frac{\bar{z} \cdot \sqrt{n-1}}{s}$ follows the t-distribu-

on with $\vartheta = n - 1$ degrees of freedom.

Table II. *Effect of phentolamine on mean blood pressure in 16 patients with heart failure.*

No.	Name	BP before phentolamine	BP after phentolamine	Time
		mm Hg	mm Hg	min.
1	E.H.	93	68	1
2	E.N.	90	80	½
3	J.N.	83	77	1½
4	A.K.	137	103	3
5	B.P.	80	70	2½
6	H.H.	113	107	2
7	O.B.	85	83	3½
8	J.L.	107	92	4½
9	I.R.	100	78	2½
10	O.D.	97	92	4
11	P.P.	100	80	1½
12	B.M.	90	85	2½
13	H.J.	100	65	2½
14	B.H.	127	117	2
15	P.S.	117	87	½
16	E.N.	100	92	1½

$\bar{x} = 101$ $\bar{y} = 86$ $\bar{z} = 15$
$s = 10.6$ $t = 5.527$
$P < 0.001$

Table III. *Effect of phentolamine on mean pulmonary artery pressure in 16 patients with heart failure.*

No.	Name	PAP before phentolamine	PAP after phentolamine	Time
		mm Hg	mm Hg	min.
1	E.H.	66.5	58.5	4
2	E.N.	17.5	15	5
3	J.N.	44	40	$1^1/_2$
4	A.K.	26	15.5	$2^1/_2$
5	B.P.	73	56.5	4
6	H.H.	10	8	2
7	O.B.	36	29	5
8	J.L.	18	17	4
9	I.R.	22.5	15	7
10	O.D.	37	29	5
11	P.P.	48	43	5
12	B.M.	26.5	21.5	3
13	H.J.	36	27	6
14	B.H.	24.5	26	$^1/_2$
15	P.S.	11	3	3
16	E.N.	9	6	4

$\bar{x} = 31.6$ $\bar{y} = 25.6$ $\bar{z} = 6$
$s = 4.2$ $t = 5.537$
$P < 0.001$

Table IV. *Effect of phentolamine on total peripheral resistance in 16 patients with heart failure.*

No.	Name	TPR before phentolamine	TPR after phentolamine	Tim
		dynes sec. cm^{-5}	dynes sec. cm^{-5}	
1	E.H.	2654	1941	3
2	E.N.	1383	1229	
3	J.N.	2189	2031	1
4	A.K.	1610	1211	3
5	B.P.	1683	1980	2
6	H.H.	1015	961	2
7	O.B.	1078	1052	3
8	J.L.	1474	1268	4
9	I.R.	1378	1075	2
10	C.D.	1250	1186	4
11	P.P.	1816	1453	1
12	B.M.	2664	2123	2
13	H.J.	1332	866	2
14	B.H.	3075	2527	2
15	P.S.	2125	1738	
16	E.N.	1737	2298	1

$\bar{x} = 1779$ $\bar{y} = 1559$ $\bar{z} = 220$
$s = 314$ $t = 2.718$
$0.01 < P < 0.02$

RESULTS

The effect of phentolamine on mean blood pressure, mean pulmonary artery pressure, total peripheral resistance and heart rate is shown in Tables II—V. The statistical calculations show a statistically significant fall in mean blood pressure, pulmonary artery pressure and total peripheral resistance, while there is a statistically significant increase in heart rate.

The observations on cardiac output, PCV-pressure and pulmonary arteriolar resistance (Table VI) are too few to allow any statistical calculation. In these 4 patients there is no change in cardiac output; the PCV-pressure shows a fall, while pulmonary arteriolar resistance shows a fall in one patient and an increase in the other.

DISCUSSION

Phentolamine has a distinct effect on systemic circulation with a fall in blood pressure and total peripheral resistance, while there is no change in cardiac output. Phentolamine accordingly has a dilating effect on systemic blood vessels. As we have few observations on PCV-pressure, it is difficult to see if the observed fall in pulmonary artery pressure is due to a dilatation of pulmonary blood vessels, or if it is passively mediated from the pressure fall in the systemic circulation.

There was, however, a distinct time difference in the pressure fall in the systemic and pulmonary circulation, which is illustrated in Fig. 1. In this patient, as in

Table V. *Effect of phentolamine on heart rate in 16 patients with heart failure.*

No.	Name	Heart rate before phentolamine	Heart rate after phentolamine	Time
1	E.H.	100	132	1
2	E.N.	60	66	1/2
3	J.N.	120	140	1 1/2
4	A.K.	120	152	3
5	B.P.	92	104	2 1/2
6	H.H.	106	106	2
7	O.B.	88	100	3 1/2
8	J.L.	—	—	—
9	I.R.	92	104	2 1/2
10	O.D.	75	75	4
11	P.P.	84	92	1 1/2
12	B.M.	80	80	2 1/2
13	H.J.	106	194	2 1/2
14	B.H.	110	110	2
15	P.S.	52	54	1/2
16	E.N.	58	62	1 1/2

$\bar{x} = 89.5$ $\bar{y} = 104.7$ $\bar{z} = -15.2$
$s = 22.0$ $t = -2.582$
$0.02 < P < 0.05$

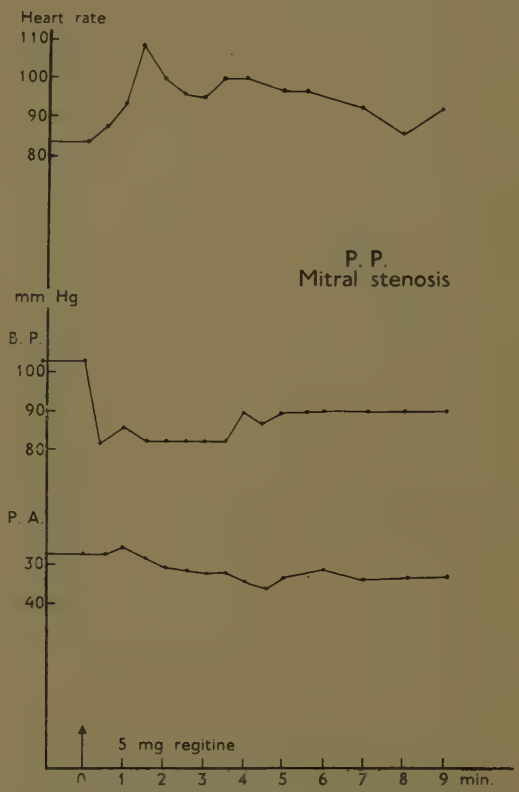

Fig. 1. Time relations between changes in heart rate, mean blood pressure and mean pulmonary artery pressure in patient No. 11.

Table VI. *Effect of phentolamine on cardiac output, PCV-pressure and pulmonary arteriolar resistance in 4 patients with heart failure.*

No.	Name	Cardiac output Before	Cardiac output After	PCV Before	PCV After	Pulm. art. resistance Before	Pulm. art. resistance After
		liters/min.		mm Hg		Dynes sec. cm^{-5}	
10	O.D.			17	7		
12	B.M.	2.7	3.2	–	–	–	–
14	B.H.	3.3	3.7	–	–		
15	P.S.	4.4	4.–	3	1.5	147	40
16	E.N.	4.6	4.2	6	2	55	78
Mean		3.75	3.78				

other patients with mitral stenosis, there was a rapid fall in systemic blood pressure during the first ½ minute. A little later the heart rate increased, while the pulmonary artery pressure fell successively during the first 5 minutes. It is difficult to assume a time difference in the reactions of the systemic and pulmonary blood vessels to phentolamine. The retardation of the pressure fall in the pulmonary artery is, however, easily explained when we take into account the heart rate.

In patients with mitral stenosis, an increase in heart rate, will, by shortening the diastolic filling time, adversely affect the passage of blood through the stenotic mitral valves. In order to maintain the blood flow, the pressure in the pulmonary circulation has to increase. This important point in the hemodynamics of mitral stenosis has been overlooked by many earlier workers in this field. It has recently been stressed by Aitchison, Cranston & Priest (1955) in their work on the effect of hydrallazine on the pulmonary circulation in mitral disease. In this work they observed a pressure rise in the pulmonary artery at the same time as the systemic blood pressure showed a distinct fall. This pressure rise in the pulmonary artery, they were able to show, was due to the accompanying increase in heart rate, as there was a close correlation between rise in pulmonary artery pressure and in heart rate. Following hydrallazine there was also an increase in cardiac output, but in this instance there was no correlation to the rise in pulmonary artery pressure. As we have mentioned earlier, Mackinnon *et al.* (1956) partly ascribed the pressure rise they observed in the pulmonary artery in one patient with mitral stenosis following priscoline the accompanying increase in heart rate.

When studying the effect of vario agents on the pulmonary circulation, it important to remember that it is a low pr sure system, with pressure only ⅛—⅙ the pressure in the systemic circulation. T pulmonary circulation, therefore, is eas influenced by changes in pressure and fl in the systemic circulation, as we fou studying hexamethonium bromide, and changes in heart rate, as shown in this stu

In order to obtain conclusive support the theory of active vasodilators in the p monary circulation, one has to use age which leave cardiac output and heart r unchanged (de Burgh Daly, 1956). Up the present there is, so far as we know, conclusive report on such an agent affecti the pulmonary circulation.

Oxygen deficiency has a constricting a oxygen supply a dilating effect on the p monary blood vessels (v. Euler & Lil strand, 1946). It has been maintained th the anoxic pressure rise in the pulmona artery was due to the accompanying increa in cardiac output. Cournand, however, in latest experiments on unilateral hypoxia man (1956), was able to confirm the c stricting effect on the pulmonary blood v sels of reduced oxygen content in inspi air. Rearranging my data from experimen anoxemia in man (Storstein, 1952), I fou that, though in most individuals there was the same time an increase in cardiac outp and pulmonary artery pressure, some in viduals showed at the same time a rise pressure, but fall in cardiac output, s porting the active vasoconstricting effect anoxia (Storstein, 1957).

SUMMARY

n 16 subjects with various heart diseases, tly mitral stenosis, phentolamine gave atistically significant fall in systemic and nonary artery pressure and in total peri- ral resistance, while there was a signi- nt increase in heart rate. The cardiac out showed no change. There were too observations to make any definite con- ions as to the effect on pulmonary arte- ar resistance.

There was a distinct time difference be- en the reactions in the systemic and pul- nary circulations. This time difference, found, was due to the effect on the heart , which, by shortening the diastolic filling e in patients with mitral stenosis, will rd and modify the pressure fall in the nonary artery.

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EFFECT OF SERUM ON SPECTRAL ABSORPTION OF BROMSULPHTHALEIN AFTER INTRAVENOUS INJECTION OF HEPARIN[1]

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Since Hahn in 1943 discovered that intravenous injection of heparin into lipemic dogs produces a clearing of the lipemic plasma, this phenomenon has been the subject of detailed investigation. During some studies on the excretion of bromsulphthalein (BSP) in man I discovered that intravenous injection of small doses of heparin under certain conditions produces changes in the spectral absorption curve for this dye, a phenomenon which may in some way be connected with the aforementioned clearing of lipemic plasma.

As this change in the absorption curve for this or other dyes does not seem to have been described in the literature, some preliminary investigations are reported below.

METHOD

All the absorption curves were determined by means of a Zeiss-Ikon spectrophotometer, model M4Q, on the following mixture: 0.5 ml of a 10 mg 100 ml stock solution of BSP (Hynson, Westcott & Dunning) + 1 ml of the plasma or serum under test + a sodium-hydroxide solution of varying concentration to make a final volume of 10 ml.

Human serum albumin (from the State Serum Institute, Copenhagen) was studied in the same manner with 1 ml of a 5 per cent aqueous solution instead of plasma or serum.

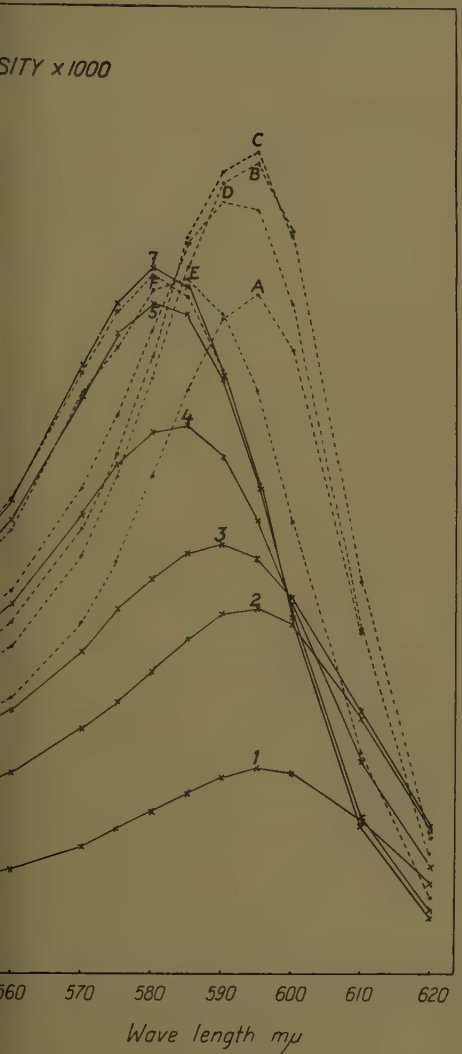
The same mixtures were used as blanks, on with 0.5 ml of distilled water instead of 0.5 ml the BSP stock solution.

RESULTS

Comparison of the spectral absorption BSP at various pH levels in mixtures co taining (1) serum (pre-heparin serum), (2 heparinized plasma, and (3) serum wit drawn after a preceding intravenous hepar injection (post-heparin serum). — The e periments were performed on five health male subjects aged 20—30 years. After wit drawal of two blood samples, one witho stabilization (pre-heparin serum), the oth stabilized with one drop of Heparin LE containing 5000 I. U./ml to 10—20 ml blood, an intravenous injection of 2—50 m of heparin (1 mg = 100 I. U.) was give Blood to which no heparin was added w then withdrawn from a vein in the opposi arm 3 minutes later (post-heparin serum The subjects were not fasting, but the blo samples were taken 2—4 hours after the usual morning meal.

The absorption of all the samples was th studied as described above at various NaO concentrations in mixtures containing (pre-heparin serum, (2) heparinized plasm and (3) post-heparin serum.

[1] Aided by a grant from the Vera and Carl Johan Michaelsen Foundation.



. Spectral absorption curves for pre-heparin mixtures (1—7) and post-heparin serum res (A—F). The final NaOH concentrations mixtures were as follows: A and 1: 0.0017 N, 2: 0.0034 N, C and 3: 0.0051 N, D and 4: 0068 N, E and 5: 0.0085 N, F: 0.017 N, 7: 0.034 N.

e absorption curves from a typical ex- ient are shown in Fig. 1. The final H concentrations in the mixtures are stated below the figure. Absorption curves for mixtures with heparinized plasma are not shown as they were identical with curves 1—7 for the mixtures with pre-heparin serum.

It is seen that at low NaOH concentrations there was a pronounced difference between the absorption curves for mixtures containing pre-heparin serum and the corresponding mixtures to which post-heparin serum was added. In either case, decreasing NaOH concentrations resulted in a shift of the peak of the curve from 5800 to 5950 Å, but the color intensity decreased in the pre-heparin serum mixtures whereas it increased initially in the mixtures to which post-heparin serum was added.

The change was uniform for all the subjects and occurred after a heparin dose of only 2 mg. It was not determined whether even smaller doses of heparin would be able to produce the same change.

In two cases pronounced turbidity occurred so rapidly at the highest NaOH concentrations (0.017 N and 0.034 N, respectively) that the entire absorption curve could not be determined at these or higher NaOH concentrations. Turbification occurred only in the post-heparin serum mixtures, but not in the corresponding mixtures containing heparinized plasma or pre-heparin serum.

Influence of serum albumin on the absorption curve for BSP. — It is known that binding of dyes to albumin may result in a shift of the peak of the absorption curve for bilirubin (With, 1945; Martin, 1949) and various other dyes (Klotz, 1946). The absorption curve for BSP was studied as described above in mixtures containing 1 ml of a 5 per cent aqueous solution of human

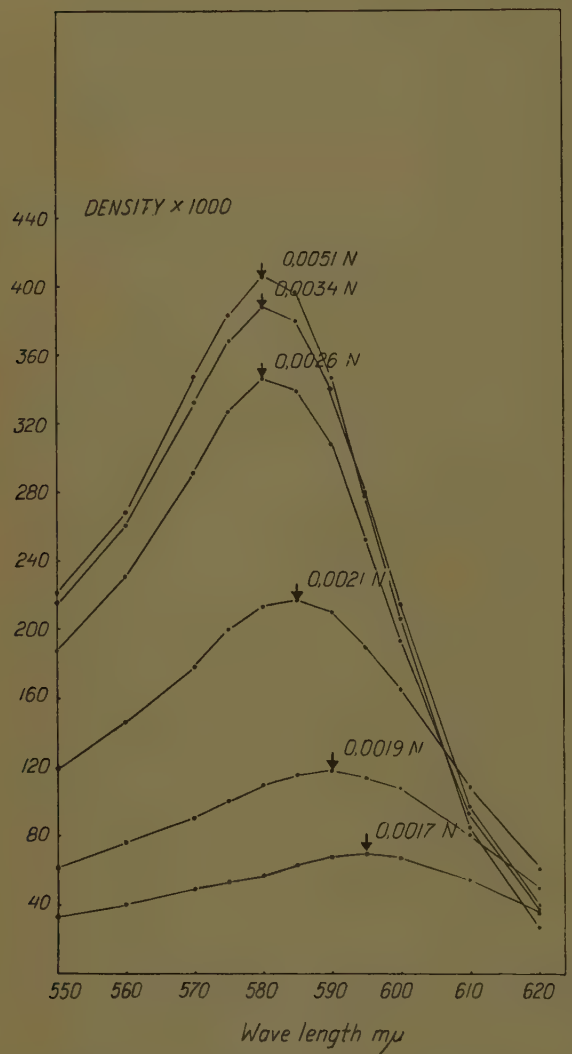


Fig. 2. Spectral absorption curves for mixtures containing human serum albumin. The NaOH concentrations of the mixtures are stated for each curve.

serum albumin. The result appears from Fig. 2. It is seen that a shift of the peak of the absorption curve from 5800 to 5950 Å occurred at decreasing NaOH concentrations. With human γ-globulin no shift of peak occurred but other serum protein fr tions have not been studied.

DISCUSSION

The shift of the peak of the absorpti curve for BSP from 5800 to 5950 Å w decreasing NaOH concentrations may pr ably be explained by a binding of the B to the serum albumin, since the latter, shown, may produce the same shift, a since, as has been shown by Ingelfing Bradley, Mendeloff & Kramer (1948), greater part of the BSP in the serum bound to the albumin fraction.

The increased color intensity of the B at low NaOH concentrations in the po heparin serum mixtures may possibly be plained on the basis of our present-d knowledge on the clearing of lipemic plas after intravenous injection of heparin.

Injection of heparin is promptly follow by the disappearance of the chylomicra p sent in the blood (Hahn, 1943). An int venous injection of only 1 mg of hepa into human beings is just as effective causing this disappearance as larger do (Grüner, Hilden & Hilden, 1953). The solution of the chylomicra occurs by a hyd lysis of their triglycerides under formati of free fatty acids (Shore, Nichols & Fr man, 1953). The free fatty acids are bou to the serum albumins (Robinson & Fren 1953; McDaniel & Grossman, 1955). Som times liberation of the fatty acids from albumins, or formation of fatty acids in large amounts that the albumins cannot b them, results in returbification, probably cause of the formation of calcium soaps w the fatty acids (Brown, Boyle & Anfins

53; Grossman, Stadler, Cushing & Palm, 55).

Accordingly, the change demonstrated in absorption curve for BSP might then be plained as follows. The change might be e either to a simultaneous binding of fatty ds and BSP to the albumin molecules or, ssibly, to the binding of the BSP to an- ier protein when the albumin is "blocked" the fatty acids. It is known that organic ds may displace dyes from serum albumin a competitive action (Klotz, 1946). The idly occurring turbification which was n in two cases might possibly be due to a rticularly high concentration of fatty acids these cases, with liberation of these from albumin molecules at the high NaOH ncentrations and formation of calcium ps. Incidentally, turbification was ob- ved in all cases, but only when the samples d been left standing for some time.

According to this explanation no effect on absorption curve can be expected on ad- ion of heparin *in vitro*, as the chylomicra not influenced by heparin under such ditions.

The phenomenon requires further investi- ion. If a displacement of BSP from the umin molecules occurs, and if similar con- ons may be demonstrated in other dyes, ection of heparin may be conceived to rt an influence on the transport and ex- tion of dyes by the organism.

SUMMARY

A change was demonstrated in the spectral orption curve for bromsulphathalein on lition of serum withdrawn 3 minutes after ravenous injection of from 2 to 50 mg of heparin into healthy male subjects. A possible explanation of the phenomenon is discussed.

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A STUDY OF THE pH AND BUFFER CAPACITY OF BLOOD, PLASMA AND RED BLOOD CELLS

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(Received for publication December 21, 1956)

The buffer capacity of a system measures its ability to maintain a certain level of acidity or alkalinity when acids or bases are added to it. In the case of blood, the buffer capacity plays an important part in the regulation of the acid-base balance of the body.

It has been shown that the component of the blood that exerts the greatest buffering action is the hemoglobin contained in the red corpuscles (Kilpi, 1952). When the hemoglobin content of the blood is 16 g/100 ml, its share of the total buffer capacity is about 79 per cent (Kilpi, 1952; Viikari, Harjola & Maamies 1954). When the hemoglobin content is reduced by hemorrhage or an operation, the buffer capacity of the blood will also decrease (Viikari, 1954). On the other hand, unexpectedly high buffer capacities have been recorded for anemic patients suffering from various internal diseases (Viikari, Harjola & Maamies, 1954). It has also been observed that a decrease in the buffer capacity and hemoglobin content of the blood of a patient after an operation is accompanied by a shift of the reaction of the blood in the alkaline direction from the value before the operation (Viikari & Salenius, 1956).

Plasma is also known to contribute, although to a lesser extent than the red blood corpuscles, to the buffer capacity of the blood (Henderson, 1928; Kilpi, 1952). The buffering capacity of the plasma is mainly determined by its bicarbonate and protein content. When the hemoglobin content was 16 g/100 ml in the blood examined by Kilpi, 21 per cent of the total buffer capacity was due to the plasma (Kilpi, 1952; Viikari *et al.* 1954). It would be interesting to know whether the part of the buffer capacity due to plasma rises when the hemoglobin content falls as a result not only of hemorrhage but also of the subsequent dilution of the blood by either tissue fluids or infusions. It may be assumed that the factors that influence the buffer capacity of the plasma will also be of importance in determining the buffer capacity of whole blood. Components of the plasma that may be involved are the bicarbonate and proteins normally present, but also components introduced by infusion.

MATERIAL

Earlier investigators have mostly followed the variation of the buffer capacity in one and the same patient before and after hemorrhage or an operation. Because so many factors that may alter the buffer capacity of the blood are involved in operations that it is difficult to control them,

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tudy the pH values and the buffer capacities e whole blood, plasma and red blood cells measured on samples taken from patients who ot been operated upon. The 30 subjects of study were patients from the surgical wards en the ages 25 and 70 years. The Hb-values l greatly in these patients. The number of samples examined was 32.

METHODS

e buffer capacities of the samples were deter-l by the potentiometric titration method of (1952). The pH values were measured poten-trically using a glass electrode. The potentio-r could be read to the nearest 0.002 pH unit. cetate buffer with pH 6.50 was employed as ence solution; the potentiometer was checked st this solution before and after each deter-tion.

he determinations were made on 5 ml volumes e undiluted samples of whole blood, plasma red blood cells. Care was taken to prevent samples from coming into contact with air. determinations were made in a thermostat at C immediately after drawing the samples which kept covered with liquid paraffin. The plasma obtained by centrifuging whole blood under l paraffin 10—15 min. at 3000 r. p. m. in ordi-alkali reserve tubes about 3 cm in diameter. mass of red blood corpuscles remaining after plasma had been removed was employed for etermination of pH and buffer capacity of the lood corpuscles.

hen the acid dissociation constants (K_i) and ntrations (C_i) of the components of an acid-mixture are known, the buffer capacity .) of the mixture can be calculated after the values have been measured before and after ddition of a known amount of acid from van 's equation:

$$\frac{d\,(B)}{d\,pH} = B.C. = 3 \cdot \sum_i \frac{K_i C_i [H^+]}{(K_i + [H^+])^2} + [H^+] + [OH^-]$$

he acid added to the 5 ml samples in the pre-investigation was 0.5 ml of 0.1 ml N HCl. The ula can then be written (Viikari, 1954):

$$B.C. = \frac{0.01}{pH_1 - pH_2}$$

For checking the method, four samples were drawn from a patient before and 1, 3 and 5 minutes after a blood pressure cuff with a pressure of 100 mm of mercury had been applied to his arm. No differences were observed in the pH values and buffer capacities determined for these four samples. Successive determinations of the buffer capacities of two blood samples yielded the following results: sample I: 0.0333, 0.0336 and 0.0317, sample II: 0.0373, 0.0368 and 0.0382. The standard deviation of a single determination computed from these values is 0.0008. No change occurred in the buffer capacity of a sample which was stored six hours in a refrigerator, but in this study the determinations were made immediately after samples had been withdrawn.

RESULTS

Whole blood

The results of the present study strongly support the opinion presented in previous studies that the buffer capacity of the blood is largely determined by its hemoglobin content. The mean hemoglobin content (Hb) of the whole blood samples analysed was 11.7 ± 2.2 g/100 ml and the mean buffer capacity (B. C.) 0.0318 ± 0.0035. The lowest hemoglobin value recorded was 6.7 g/ 100 ml and the highest 15.5 g/100 ml. The corresponding buffer capacities were 0.0250 and 0.0377. The value of the association coefficient Q illustrating the relationship between hemoglobin content and buffer capacity was found to be + 0.76 and the linear correlation coefficient r (the product moment coefficient of correlation) was found to be + 0.79.

The calculation of the association coefficient Q and the linear correlation coefficient r has been done according to Yule & Kendall. The association coefficient indicates whether there is any correlation between the two variables and whether this is positive or negative. The linear correlation coefficient

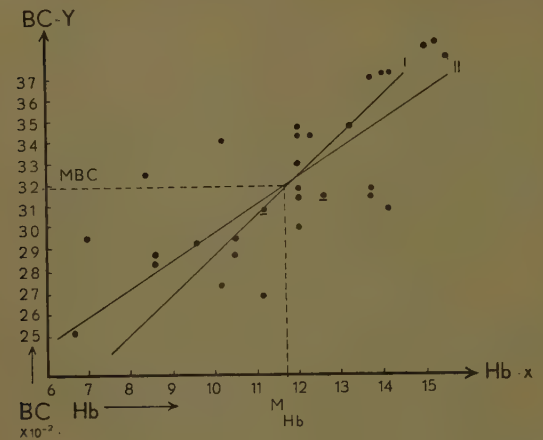


Fig. 1. Correlation between buffer capacity and hemoglobin content of whole blood. Mean Hb: 11.7 ± 2.2 g/100 ml. Mean B. C.: 0.0318 ± 0.0035. Linear correlation coefficient r = + 0.79. The underlined values were recorded twice.

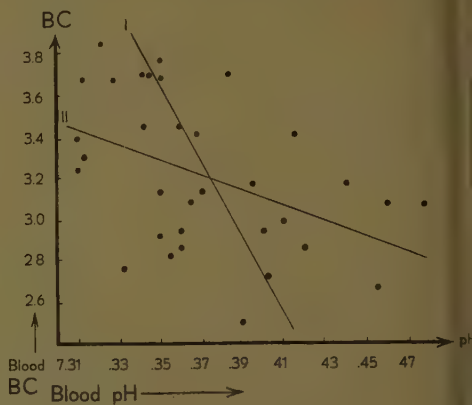


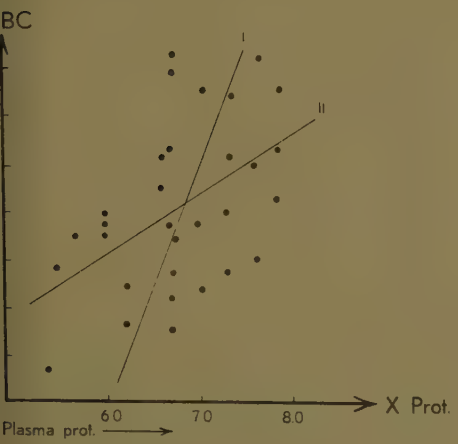
Fig. 2. Correlation between buffer capacity and p of whole blood. Mean pH: 7.374 ± 0.042. Me B. C.: 0.0318 ± 0.0035. Linear correlation coefficie r = — 0.45.

(product moment coefficient of correlation), on the other hand, indicates the absolute value of positive or negative linear correlation between the variables. This coefficient may have any value between —1 and +1. Absolute values of r in the range from 0.00 to 0.30 are generally considered to imply a weak correlation, values between 0.30 and 0.60 moderate correlation, values between 0.60 and 0.80 fairly strong correlation and values higher than 0.80 a strong correlation. Values exceeding 0.80 are relatively rare in practice (Vahervuo, 1952). The value +0.79 hence indicates that practically speaking a strong correlation exists between the hemoglobin content and buffer capacity.

With sufficient data, it might be possible to compile a table from which the buffer capacity of the blood could be read when the hemoglobin content is known.

The relationship between hemoglobin content and buffer capacity can also be expressed graphically by means of regression lines. Th equations for the regression lines are in th present case: I: X = 5.33Y — 5.25 and I Y = 0.13X + 1.66 when X = Hb an Y = BC. Fig. 1.) If these lines had coi cided, the correlation between the two va ables would have been perfect. This is, ho ever, very unusual in the case of biologic data. If no correlation existed between t two variables, the two lines would have inte sected at right angles.

The coefficients for the relationship b tween the pH and buffer capacity of wh blood are: Q = —0.86 and r = —0.45. T regression lines are drawn in Fig. 2. T mean pH of the blood samples was 7.374 0.042, the lowest value 7.310 and the high value 7.478. The mean and the range of t buffer capacity values were given above. T values of the coefficients reveal that the bu fer capacity of the blood increases with t acidity. The linear correlation coefficient



3. Correlation between blood buffer capacity serum protein content. Mean B. C.: 0.0318 ± 35. Mean serum protein content: 6.8 ± 0.7 per nt. Linear correlation coefficient r = + 0.48.

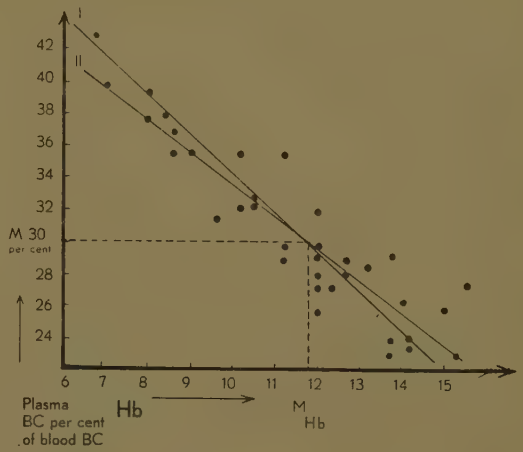


Fig. 4. Correlation between percentage of the blood buffer capacity due to plasma and blood hemoglobin content. Mean buffer capacity percentage: 29.98 ± 4.88 per cent. Mean hemoglobin content: 11.7 ± 2.2 g/100 ml. Linear correlation coefficient r = — 0.90.

a low value, and it may be that the cor-
tion is not linear.

The coefficients for the correlation be-
en the buffer capacity of the blood and
ım protein content are Q = +0.64 and
+0.48. The regression lines are drawn
Fig. 3.

For the relationship between blood buffer
acity and plasma bicarbonate, Q was
ıd to be +0.10. This value is so low that
e is no point in giving the value of the
ar correlation coefficient. For the rela-
ship between blood buffer capacity and
cell volume, Q = +0.34.

he mean pH of the blood was 0.048 pH
higher than the meam plasma pH and
1 pH unit lower than the mean red cell
Similarly, the mean blood buffer capacity
0.0166 unit higher than that of the
ma and 0.0334 unit lower than that of
red cells.

Plasma:

The mean buffer capacity of the plasma is thus seen to be considerably lower than the buffer capacities of the whole blood and the red cells. The plasma is the most alkaline of the three. It has been previously found (Henderson, 1928; Kilpi, 1952) that the plasma is responsible for about 21 per cent of the buffer capacity of whole blood when the hemoglobin content is 16 g/100 ml. In the present study the part of the whole blood buffer capacity due to plasma has been computed by multiplying the originally measured plasma buffer capacity by (100 — blood red cell volume per cent) per cent. This value has been taken as the relative plasma buffer capacity and its share of the whole blood buffer capacity has been calculated as a percentage. The mean of the values calculated in this way was 29.98 ± 4.88 per cent.

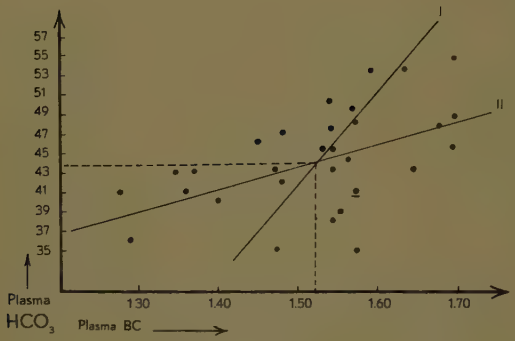


Fig. 5. Correlation between buffer capacity and alkali reserve of plasma. Mean alkali reserve: 44.0 ± 4.88 vol. CO_2 per cent. Mean plasma B. C.: 0.0152 ± 0.0011. Linear correlation coefficient r = + 0.49.

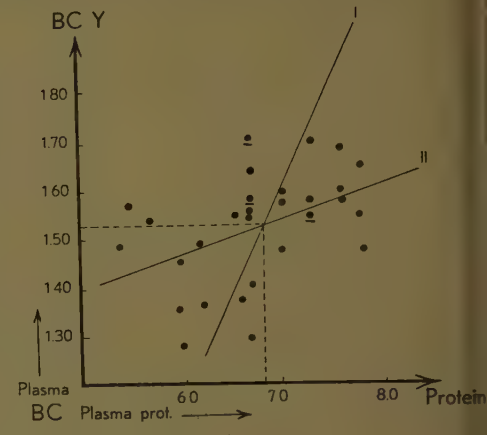


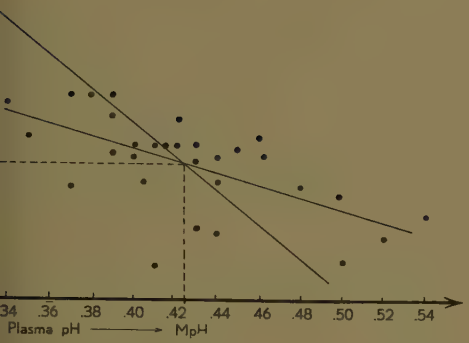
Fig. 6. Correlation between buffer capacity serum protein content of plasma. Mean pla B. C.: 0.0152 ± 0.0011. Mean serum protein tent: 6.8 ± 0.7 per cent. Linear correlatio coefficient r = + 0.39.

The lowest value was 22.9 per cent for a hemoglobin content of 15.2 g/100 ml. The highest value, 42.8 per cent, was found for a blood sample with a hemoglobin content of 6.7 g/100 ml. The value of the association coefficient Q for the relationship between the plasma buffer capacity percentage and the hemoglobin content was —0.98 and that of linear correlation coefficient (r) —0.90. The regression lines are shown in Fig. 4. The part of the blood buffer capacity due to plasma thus varies almost linearly and inversely as the hemoglobin content of the blood.

The value of the association coefficient Q for the correlation between plasma buffer capacity and plasma bicarbonate is +0.70 and that of the linear correlation coefficient (r) + 0.49. The regression lines are drawn in Fig. 5. The highest plasma buffer capacity measured was 0.0169 and the lowest 0.0128, the mean value being 0.0152 ± 0.0011. The extreme alkali reserve values were 54 per cent CO_2 corresponding to 23.5 meq/1 35 per cent CO_2 corresponding to 15.1 me The mean of the alkali reserve values 44.0 ± 4.88 per cent CO_2.

The value of the association coefficien for the correlation between plasma bu capacity and serum protein content + 0.66, and that of the linear correla coefficient (r) + 0.39. The regression l are shown in Fig. 6. The buffer capa values were given above. The highest pro content was 7.8 per cent and the lowest per cent, the mean content 6.8 ± 0.7 per

As already mentioned, the mean pla pH was 0.048 pH unit higher than the n whole blood pH. The mean plasma pH 7.422 ± 0.046, the highest recorded v 7.540 and the lowest 7.340. For the cor tion between plasma pH and plasma bu capacity, Q = — 0.72 and r = — 0.61. conclusion is that the buffer capacity of plasma is greater the lower the pH.



. 7. Correlation between buffer capacity and pH
lasma. Mean plasma BC: 0.0152 ± 0.0011. Mean
plasma pH: 7.422 ± 0.046. Linear correlation
coefficient r = —0.61.

Red cells.

The mean pH of the red cells was 7.320,
ich is 0.054 pH unit lower than the mean
ole blood pH and 0.102 lower than the
an plasma pH. The lowest pH recorded
the red cells was 7.250, the highest 7.448.
e mean buffer capacity of the red cells,
652, was appreciably higher than the mean
ffer capacities of whole blood and plasma.
e highest buffer capacity measured for the
l cells was 0.08833, and the lowest 0.0495.
For the correlation between the pH and
ffer capacity of the red cells, Q = —0.24.
r the correlation between blood hemoglobin
ntent and red cell buffer capacity, Q =
0.11, and between the buffer capacity and
ative volume of the red cells, Q = + 0.40.

DISCUSSION

The results of the present study of the
dity and buffer capacity of whole blood,
sma and isolated red blood cells show
arly, as was expected, that the buffer
capacity of whole blood is closely connected with its hemoglobin content. The serum proteins are also responsible for some of the buffer capacity of the blood; this may be important when the hemoglobin concentration is low.

According to Kilpi, hemoglobin exerts its greatest buffering action when the pH is 7.30, this action becoming weaker the more the pH deviates from this value in the acid or alkaline direction. The blood samples of the present series were for the most part more alkaline than pH 7.30, their mean value being 7.374 ± 0.042 and the range 7.310 — 7.478. As expected, the buffer capacities had the highest values when the pH values were close to 7.30. Blood is not, however, solely a solution of hemoglobin, but a mixture of cells and plasma, and the buffer capacity of the mixture is influenced also by other factors in addition to hemoglobin content. This was evident already in the results of Kilpi, who found that the buffer capacity of the blood is higher at pH 6.50 than at 7.30 although the buffer capacity of hemoglobin is considerably lower at the former pH. It is thus obvious that regardless of the hemoglobin content the buffer capacity of the blood increases as the acidity increases, at least within certain limits. A possible explanation for this may be that as the hemoglobin content of the blood diminishes, the blood becomes diluted with more alkaline tissue fluids (plasma) which have a greater effect on the acidity owing to the decreased buffer capacity (Kilpi 1956).

The results of the present study indicate that the relative significance of the plasma as a buffering component of the blood increases, and furthermore almost linearly, as

the hemoglobin content is lowered. This is noteworthy since in anemic conditions such as those resulting from hemorrhage or operation, the factors that determine the buffer capacity of the plasma attain a relatively greater importance. In addition to bicarbonate and proteins that normally function as buffering agents in plasma, components that are introduced into the blood in infused solutions may also greatly influence the acid-base equilibrium.

As in the case of whole blood, also the buffer capacity of the plasma was found to be connected with the acidity level in that a more acid plasma tended to have a greater buffering action. An explanation for this may be that according to Kilpi the buffering action of bicarbonate in the plasma increases as the plasma becomes more acid. The increase in buffer capacity due to bicarbonate observed when the pH changed from 7.30 to 6.50 is 0.0079 (Kilpi, 1952). Also other plasma components may play a part in determining the buffer capacity at acid pH values.

As expected beforehand, the variations in the bicarbonate and protein contents were found to exert a marked influence on the buffer capacity of the plasma. Concerning bicarbonate, it is true that the ratio $H_2CO_3/-$ determines the buffer capacity and not the bicarbonate content alone. In this investigation only the bicarbonate was determined, it being assumed that the ration was the normal 1 : 20.

The acidity and the buffer capacity of the red blood cells did not appear to follow the variations of the contents of the components investigated.

SUMMARY

The acidities and the buffer capacities whole blood, plasma and isolated red blo cells have been measured in 32 blood sampl The hemoglobin contents of these blo samples, which have been taken for the m part from surgical patients who had r been operated upon, varied from 6.7 g 15.5 g/100 ml.

The acidities were measured potentiom rically and the buffer capacities by potenti metric titration at 38° C in the absence air. The results revealed that hemoglobin the most important buffering component whole blood, although the plasma protei contribute in a lesser degree to the buff capacity. The relative importance of t plasma in determining the buffer capacity blood increased as the hemoglobin conte diminished. The buffering action of bo the blood and plasma increased with t acidity of the sample. None of the measur values was found to be correlated with t acidity and buffer capacity of the red ce isolated from the blood by centrifugation.

Of the components of the blood, the plas was more alkaline than whole blood or c centrifugate. The buffer capacity was great in the cells and lowest in the plasma.

All the results are shown together w statistical data in the figures.

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HAPTOGLOBIN IN PERNICIOUS ANEMIA

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Haptoglobin (Hp) is a term introduced by Jayle (1939) to designate a group of serum mucoproteins capable of binding hemoglobin stoechiometrically *in vivo* and *in vitro*. The haptoglobin content of the serum can be determined by two methods, both based on the affinity of haptoglobin for hemoglobin. One of the methods is titrimetric and utilises the peroxidatic properties of the Hp-Hb complex (Jayle, 1940). The other method (Laurell & Nyman, 1956) is based on the difference

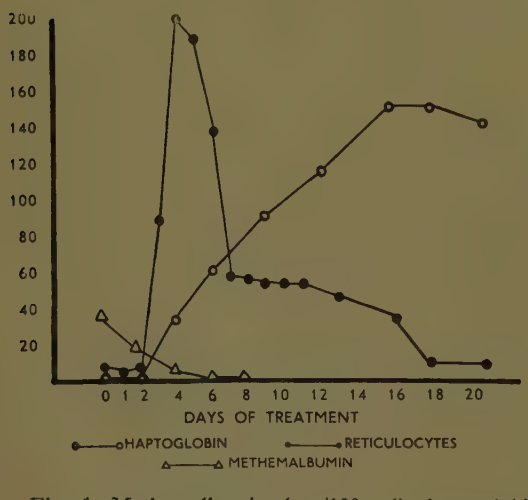

Fig. 1. Methemalbumin (mg/100 ml), haptoglobin and reticulocytes (per thousend red cells) in a case of pernicious anemia before and during treatment with vitamin B_{12}.

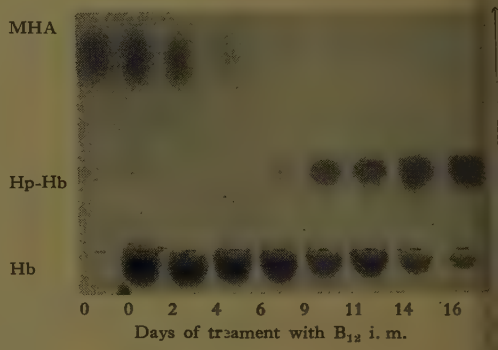

Fig. 2. Methemalbumin and haptoglobin in a t of pernicious anemia during remission. Electrophoresis in phosphate buffer pH 7, color with leucomalachite green and hydrogen perox Sample 1 without addition of Hb. Samples 2—9 after addition of 0.75 mg Hb/ml serum

observed in the electrophoretic mobility the Hb-Hp complex and that of the f hemoglobin. Haptoglobin migrates with α_2-fraction (of which it normally represe about 20 per cent), free hemoglobin, w the β-fraction, and the Hp-Hb complex, tween the α_2 and β-fractions. According this method, the haptoglobin content is m sured by determination of the largest amo of Hb that can be bound by the α_2-fract without the appearance of any free Hb the β-fraction.

n none of the sera from 8 patients with reated pernicious anemia (histamine re- ctory achylia, megoblasts in the bone mar- and favourable response to vitamin B_{12}) there any hemoglobin demonstrable but of them hemochromogen was found to ound to the albumin fraction (methemal- in). In none of the 8 sera was there any toglobin demonstrable. Treatment with min B_{12} produced first a successive de- se in the electrophoretic methemalbumin e (parallel with a decrease in the total ochromogen content as determined by zidine according to Crosby). Within 3—6 s of the beginning of treatment (approxi- mately simultaneously with the disappear- ance of the methemalbumin) traces of hapto- globin are seen which afterwards gradually increase to reach normal level within 2—3 weeks.

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RECTAL ABSORPTION OF BENZYLPENICILLIN

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Previous investigations have shown that therapeutically effective serum concentrations can be attained by rectal administration of benzylpenicillin sodium in a suppository (Loewe, Alture-Werber & Rosenblatt, 1945; Mandel & Thayer, 1948; Gaida & Neumeyer, 1951; Rudelius, 1953), the absorption, however, being very incomplete. Experiments by Gundersen (1948) called attention to the possibility of increasing absorption of benzylpenicillin from a rectal suppository, by incorporating sodium lauryl sulfate in the suppository. Further experiments in rabbits by the author (Backe-Hansen, 1957) showed that sodium lauryl sulfate increased the absorption of benzylpenicillin from suppositories (suppository base being cocoa butter or Imhausen Base H) 4 to 5 times on an average, the resulting serum concentrations being equal to those following an intramuscular injection. The increased absorption was ascribed to increased release of benzylpenicillin from the suppository base, prevention of penicillinase activity, and possibly an effect on the rectal mucosa resulting in increased permeability.

In the present investigation suppositories containing 500 0000 units of benzylpenicillin sodium and 0.1 g of sodium lauryl sulfate were inserted rectally in human adults. At intervals up to 2 hours the penicillin ser concentrations were determined, and values obtained were compared with serum concentrations following intramus lar injections of benzylpenicillin in the sa individuals.

EXPERIMENTAL

Suppositories of the following composition w prepared by moulding:

Benzylpenicillin sodium (Penicillin A.L.)	500 000 u
Sodium lauryl sulfate	0.10 g
Imhausen Base H	1.75 g

Imhausen Base H is a fatty suppository b partly synthetic, and in technical respects super to the traditional cocoa butter. It is reported consist of a triglyceride with a small amount o monoglyceride added as emulgent.

MATERIAL AND METHODS

Clinical test

The suppository, containing 500 000 units benzylpenicillin sodium, was inserted in the morni Blood samples were withdrawn after 15 minu 30 minutes, 1 hour and 2 hours. On the next an equal penicillin dose, in aqueous solution, injected intramuscularly, and blood samples w withdrawn at the same intervals.

The blood samples were centrifuged, kept i refrigerator at 4° C and analyzed within 24 ho

The patients were bedridden, of both sexes, range from 35 to 77 years (average 59 years). a rule the suppositories were well retained, but a few cases (not tabulated) defecation occur shortly after insertion of the suppository.

Table I. *Penicillin serum concentrations in units/ml after rectal and intramuscular administration, respectively, of 500 000 units of benzylpenicillin sodium:*

tient	Time after dosage:			
Rectal	15 minutes	30 minutes	1 hour	2 hours
1	2.6	3.9	4.5	8.0
2	0.7	0.7	0.7	0.6
3	1.9	1.6	1.2	0.7
4	1.4	2.1	1.1	ca. 0.1
5	3.1	4.4	2.2	1.1
6	2.8	2.5	1.3	ca. 0.3
7	1.4	3.0	1.9	ca. 0.5
8	4.2	3.9	1.7	0.8
9	2.2	3.4	1.4	0.7
10	1.4	3.0	1.2	ca. 0.4
11	2.0	2.8	0.9	ca. 0.3
12	0.9	2.5	1.9	0.8
13	0.7	1.5	0.8	ca. 0.4
14	10.0	5.8	2.1	0.6
15	5.8	5.8	2.8	1.7
$\bar{x}$	2.74	3.13	1.71	1.13
s	2.44	1.47	0.97	1.94
e	0.63	0.38	0.25	0.49
IM	15 minutes	30 minutes	1 hour	2 hours
1	10.8	15.3	15.5	7.0
2	3.9	6.5	9.0	5.8
3	12.5	15.5	12.5	7.5
4	9.8	10.8	8.3	2.6
5	15.5	19.0	9.3	4.5
6	7.5	8.0	5.5	1.6
7	9.5	9.0	6.3	3.0
8	4.0	5.9	5.5	2.4
9	8.3	9.5	10.5	6.3
10	3.1	4.0	4.8	3.5
11	2.9	4.5	5.8	2.8
12	8.8	10.5	9.5	5.3
13	5.3	7.0	9.8	6.3
14	9.5	6.8	5.3	1.7
15	12.5	12.5	10.0	5.5
$\bar{x}$	8.26	9.65	8.51	4.38
s	3.80	4.35	3.03	1.99
e	0.98	1.12	0.78	0.51

epresents mean value, s standard deviation $\left(\sqrt{\frac{\Sigma(x-\bar{x})^2}{n-1}}\right)$

e mean error $\left(\frac{s}{\sqrt{n}}\right)$

Microbiological assay

The penicillin concentrations were determined in appropriate dilutions of serum by an agar cup method, *Sarcina lutea* ATCC 9341 being the test microbe. Substrate: Bacto Penassay Seed Agar (Dehydrated), Difco Laboratories. The technique used has been described by Trolle-Lassen (1951).

The serum samples were diluted at least 10 times with a phosphate buffer solution of pH 7, thus eliminating any protein binding (Eagle & Tucker, 1948).

RESULTS

The penicillin serum concentrations were determined in 4 blood samples from 15 patients in each series, the tests being cross-over tests.

The results are given in Table I.

DISCUSSION AND CONCLUSION

It appears from Table 1 that benzylpenicillin sodium was absorbed rapidly from the rectal suppositories. After 15 minutes the serum concentrations were considerable in most cases, and as a rule maximal serum concentrations were attained within 30 minutes.

As compared with previously published data it seems that incorporation of sodium lauryl sulfate in the suppository has resulted in increased absorption of benzylpenicillin. But generally, absorption was not promoted to the same extent as in the experiments on rabbits referred to above. In a few individuals the penicillin serum concentrations attained rectally equalled the corresponding serum concentrations following intramuscular injections, but on an average the maximal serum concentration in the "suppository" series amounted to only about ⅓ of the maximum concentration by i.m. injection.

The difference between the two series is statistically significant ($P < 5$ per cent).

SUMMARY

Benzylpenicillin sodium has been giv rectally in suppositories to 15 bedridd patients (adults). Suppository base: Imha sen H, with 0.1 g of sodium lauryl sulfa added to promote absorption. Next day t same penicillin dose was given intramusc larly. Blood samples were withdrawn aft 15 minutes, 30 minutes, 1 hour and 2 hou in both series and the penicillin concentr tions determined microbiologically in dilut serum, using an agar cup method. Te microbe was *Sarcina lutea.*

Addition of sodium lauryl sulfate to t suppository did not promote absorption benzylpenicillin in man to the same extent was found in earlier experiments with ra bits (Backe-Hansen, 1957); on an avera the maximal penicillin serum concentr tion, following insertion of a suppositor amounted to ⅓ of the average peak aft intramuscular injection.

ACKNOWLEDGEMENTS

This work was aided by a grant from A/S Fa maceutisk Industri, Oslo. My thanks are due Professor C. Müller, M. D., Head of the Medi Department VIII, Ullevål Hospital, Oslo, for kind interest, and to R. Thorsen, M. B., for c ducting the clinical tests.

Docent S. Funder, Ph. D., Head of the Bac riological Department, the Pharmaceutical Instit of the University of Oslo, kindly allowed me carry out the microbiological assays in his labo tories.

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HYPOXIA FOR PREVENTION OF PHYSIOLOGIC ANEMI IN THE NEONATAL PERIOD OF RATS

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(Received for publication February 4, 1957)

In 1954 we proposed that experiments should be performed in which newborn premature infants were kept for some time in incubators with less oxygen than air. The main reason for this proposition was to protect these infants from the consequences of the intense vascular reactions caused by the sudden changes in the oxygen and carbon dioxide tensions at birth. Another interesting aspect of keeping infants in low oxygen tension would be its possible influence on blood formation. As early as 1890 Viault proved that hypoxia produces polycytemia, but it was not until 1926 that Ziegelroth and 1930 that Goldbloom & Gottlieb proposed the theory that the polycytemia of intrauterine life is due to hypoxia and the anemia in the neonatal period is due to the cessation of the hypoxic stimulus. In order to study this hypothesis experimentally in animals, we have chosen rats, since these animals have a similar type of physiologic anemia as infants.

METHODS

Two litters of newborn rats were pooled in each experiment. Half of each litter was given to one of the mothers and the other two halves of the litters to the other mother. The mothers were alternated between the two groups daily. One group of rats was kept constantly in 15 per cent oxygen and only taken out into air for weighing, which amounted to less than half an hour daily. The other group, i. e. the control group, was kept room air. The humidity was about 70 per cent a was approximately the same for the two grou The baby rats in the two groups both behaved the same way and there was no cyanosis or i creased mortality in the ones kept in 15 per c oxygen. However, the rat mothers were sometim irritated by the low oxygen tension in the ambie air and on the first day of the experiment they not always take as much care of the small rats wh they were in 15 per cent oxygen as when th were in air. Some experiments failed because t rat mothers killed the baby rats. The newborn r used were never more than 12 hours old at t beginning of the experiments.

In the airtight cages, the desired oxygen co centration was obtained by blowing a mixture 15 per cent oxygen and 85 per cent nitrogen throu the cages. Repeated air samples taken every t showed that the oxygen concentration seldom var more than ± 1 per cent.

The hemoglobin and the hematocrit values w measured. The blood samples were collected tubes which had been prepared with heparin. the blood was taken by section of the subclav artery in the etherized animal and the animals w sacrified afterwards. The hemoglobin was det mined as oxyhemoglobin in a Beekman C pho electric colorimeter with an interference fi (Bausch & Lomb) of 575 mμ. The blood diluted one part in 240 parts of 0.01 per c Na_2CO_3 solution. To calculate the amount hemoglobin, a millimolecular weight of hemoglo was assumed to be 68 000. The determinations w carried out by the Central Laboratory of University Hospital through the courtesy of P fessor C. G. Holmberg and Dr. S. Hansson.

order to measure the hematocrit, the blood aspirated into dry glass tubes 50 mm. long with an internal diameter of 0.7 mm. The s were closed with rubber straps and centri- d at 3000 r. p. m. for 20 minutes. Depending on amount of blood available, two, three or four surements were made on the same blood and average of the results was used. If the double ing for the hematocrit varied more than 3 cent, or the double readings for the hemo- in more than 0.6 g per 100 ml, the results were ted.

RESULTS

37 animals were studied. Although the in of rats used is uniform and has been so for many years at this laboratory, hemoglobin values differed from litter to r, whereas the blood values generally ed satisfactorily within the same litter. example of this is shown in Table I. hin the same litter at the same age the ation in the hematocrit and the hemo- in values is not too great, whereas the

ble I. *Hematocrit and hemoglobin in some different litters of newborn rats.*

r	Age in days	Air		15 per cent oxygen	
		Hematocrit per cent	Hemoglobin g per 100 ml	Hematocrit per cent	Hemoglobin g per 100 ml
	5	34.1	12.2	38.0	15.6
		30.5	12.4	37.0	15.1
		30.5	11.2	36.0	13.7
	12	28.8	9.3	32.8	11.8
	12	33.0	13.6	40.7	15.4
		32.5	12.0	38.0	15.7
		30.5	11.9	37.8	15.7
	16	27.7	9.2	32.0	10.6
		28.7	9.7	33.3	10.8
		30.0	9.8	33.0	12.3

Table II. *Average hematocrit and hemoglobin values in rats reared in air and in 15 per cent oxygen.*

	Number	Hematocrit per cent	Number	Hemoglobin g per 100 ml
Air	84	28.0	86	9.3
15 per cent O_2	93	33.0	92	11.1
Mean difference		5.0		1.8

values differ more from litter to litter. As seen in Table I, the values are higher in 15 per cent oxygen than in air. In all baby rats the hemoglobin values within the same litter at the same age are higher in 52 pairs (i. e. one rat living in air and the other in 15 per cent oxygen) in the group reared in 15 per cent oxygen. In three pairs the opposite occurs and in two pairs the values are the same in both groups. In 54 cases, the hematocrit is higher in the group reared in 15 per cent oxygen than in the group kept in air and in two cases the couples have the same hematocrit. The signifance of these results is evident.

Table II gives the average results for all the animals in 15 per cent oxygen and for those in air and demonstrates the considerable difference in the blood values in the two groups.

The hematocrit in each baby rat together with the average values are given in Figs. 1 and 2. It will be observed that in the animals kept in air there is an immediate drop in the hematocrit which is more or less linear until about the 18th day. The hematocrit then remains low for some days and seems to rise again on about the 26th day. In animals kept in 15 per cent oxygen the hematocrit is main-

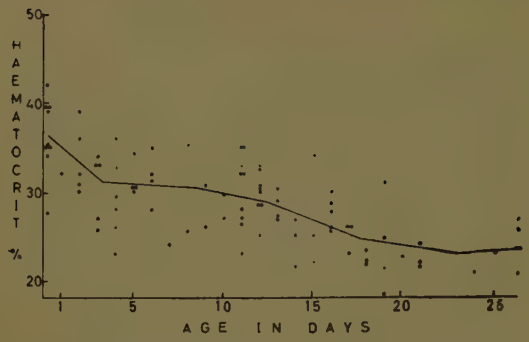

Fig. 1. Hemotocrit in per cent for 86 baby rats reared in air 1 to 26 days. Each point represents one baby rat and the continuous line the average values.

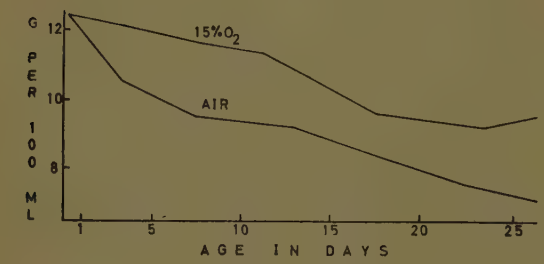

Fig. 3. Average values of hemoglobin in g per 100 ml blood for 86 baby rats reared in air and 92 baby rats reared in 15 per cent oxygen 1 to 26 days.

tained almost at the initial level for some 13 days, after which it drops. The values are low between the 19th and the 25th day after which they seem to increase. These average values for each day are invariably higher than for the animals in air. The hemoglobin values vary in the same way as the hematocrit, as shown in Fig. 3. The hemoglobin and hematocrit average values in 15 per cent oxygen are constantly and considerably higher than the values in air. It is obvious that these differences are significant.

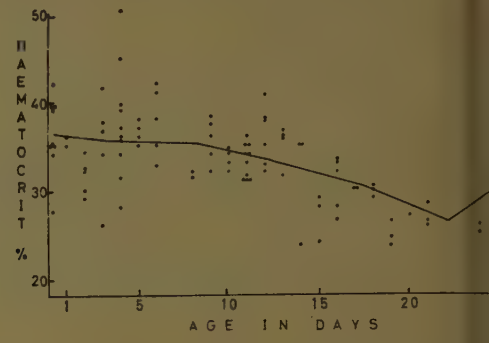

Fig. 2. Hematocrit in per cent for 92 baby r reared in 15 per cent oxygen 1 to 26 days.

DISCUSSION

Recent reviews on physiologic anemia the neonatal period have emphasized the i activity of the bone narrow in the first wee of life (Glazer, Limarzi & Poncher, 195 Gairdner, Marks & Roscoe, 1952, 195 Schulman, Smith & Stern, 1954) and c related this with the increase in oxygen te sion which occurs after the child is bor It seems to be the same reaction as is knov to occur in natives living at high altitud when they descend to sea level (Meri 1950; Lawrence *et al.*, 1952). If this hyp thesis is correct it should be possible to p vent, or at least reduce, this anemia by e posing them to an oxygen tension which lower than air. In order to obtain sufficie experimental data we had to use newbo rats which have the same type of anemia man (Contopoulus *et al.*, 1955). In fact, also found a considerable reduction in t anemia of our rats when they were expos to 15 per cent oxygen. This may seem contradict the results published by Con poulus *et al.* (1954). They intermittenly e posed 6 rats of between 4 and 18 days of a to an altitude of 15 000 ft and kept 6 cont

nals at sea level. Their conclusion was there is no decrease in the anemia of the natal period if the rats are exposed to oxia. A study of their figures, however, als that the hemoglobin was 6.7 g per ml blood in the rats at sea level and in those at 15 000 ft. There is no appreci- difference in their hematocrit values.

n his report on the influence of hypoxia actating rats on the blood of their off- ng, Grant (1955) communicates that he ormed four preliminary experiments with es of rat litters placed in a low pressure nber for 6 hours a day. The baby rats n to have been two days old when they e placed in the chamber. He found no cing difference in the blood values be- en the anoxic and the control rats. No res are published.

n the preliminary experiments, where we not maintain a constant oxygen concen- ion of 15 per cent but often had more gen in the cages, the differences between hemoglobin values in the baby rats in oxia and those in air were less evident n in the series presented above. It there- seems possible that the difference in the lts obtained by Contopoulus *et al.* and nt compared with our results may be ained by the fact that the former had intermittent hypoxia. Another, and ably equally important, factor is that they not place the young rats in the low pres- chambers before the animals were two our days old.

CONCLUSIONS

he anemia in the neonatal period of rats onsiderably delayed and lessened if the ng rats are exposed to continuous hyp- oxia after birth. In our experiments the rats were not exposed to such intense hypoxia as they were *in utero*. The experiments therefore do not indicate whether the increase in oxygen tension in extrauterine life is the sole factor in producing this anemia in the neonatal period or if there are other unknown factors of importance.

SUMMARY

92 newborn rats were reared in 15 per cent oxygen and sacrificed after 1 to 26 days. The hemoglobin and the hematocrit were studied and compared with 86 control animals reared in air.

Hemoglobin and hematocrit is higher in the animals exposed to 15 per cent oxygen. The mean difference in hemoglobin is 1.8 g per 100 ml and in hematocrit 5.0 per cent.

The results obtained support the hypothesis that the anemia in the neonatal period is due to the cessation of the stimulus for blood formation caused by the intrauterine hypoxia which occurs when the animals start breathing air.

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A STUDY OF SERUM GLYCOPROTEINS IN CANCER

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hanges in the content of serum glycoeins in various pathological conditions e been investigated by a great number of ntists. Already as early as 1885 Freund 25) reported an increased content of ohydrate in cancer tissue. Mörner (1893) mined the occurrence of protein-bound saccharides in human serum. During nt decennia, the content of serum glycoeins in cancer patients has been inigated — *inter alia* — by Lustig & Lan- (1931), Seibert, Seibert, Atno & Campell 47), Shetlar, Bryan, Foster, Shetlar & rett (1949), Weisbrod (1950), Green- (1951), Diengott (1954), Roboz, se & Forster (1955), Puls & Albaum 56), and Lockey, Andersen & MacLagan 5). The material of these investigators es in composition and quantity. For rmination of polysaccharides various ods have been used and the results have given as protein-bound hexose or hexoine. In accordance with the various ods of analysis, the results show conable variation, but compared to the ective normal material all the investirs have reported an increase of serum oproteins in cancer patients. A closer stigation regarding the grouping of the s according to the localization of cancer in different organs, operability, postoperative course and occurrence of metastases has not yet been made. The distribution of polysaccharides in different protein fractions has formerly been examined by Shetlar, Shetlar, Richmond & Everett (1950), who fractionated serum from cancer patients with salt-precipitation methods according to Milne (1947) and reported a marked increase of polysaccharides bound to albumin and a less considerable increase of α-globulin polysaccharides. Seibert, Pfaff & Seibert (1948) fractionated serum according to Cohn and found an increased amount of polysaccharides in fraction IV—6 corresponding to α_2-globulin. Weimer, Redlich-Moshin, Salkin & Book (1954) availed themselves of the low temperature — ethanol method to fractionate the proteins, and found only small quantities of carbohydrate bound to albumin. Roboz (1955) examined 8 cases of malignant tumor in the central nervous system with paper electrophoretic separation of serum polysaccharides according to Köiv & Grönwall (1952) and found an increase of polysaccharides in α_2-globulin and a decrease in β-globulin. Goa (1955) has determined the content of hexose and hexosamine in protein fractions obtained by preparative electrophoresis on filter paper.

Of three cases of cancer two had increased α_1- and α_2-globulin polysaccharides.

The aim of this investigation has been to study the changes in serum glycoproteins with respect to content and distribution in different protein fractions in cancer of varying localization, to follow the changes that take place in connection with operation or other therapy, to study the influence of metastases, and to collect a material so extensive that the results could be evaluated statistically.

MATERIAL

All the examined patients have been under treatment at the surgical and radiological clinics of the University Hospital. The samples have been taken from patients suffering from cancer, where the diagnosis had been proved by histological examination. The age of the patients varied from 28 to 87 years. Patients, however, who were treated radiologically, are not included in this report. Patients who were also suffering from other illnesses which can cause an increase of the protein-bound serum polysaccharides, were excluded. These diseases were mainly acute infections with fever, rheumatoid arthritis and diabetes with vascular changes. (For further references see Winzler (1955).) Samples were taken as soon as possible after admittance and before any treatment, and thereafter one month at the earliest after healing of the operation wound; the trauma itself causing an increased content of the polysaccharides (Shetlar, Bryan, Foster, Shetlar & Everett, 1949; Schacter, Supplee & Entenman, 1952). After operation follow-up samples were taken from the patients every month, if possible. The material has been divided according to the localization of cancer into the following groups: gastrointestinal tract, breast, prostate and other localization. Gastrointestinal cancer comprises 80 cases (98 samples): 52 in the stomach, 13 in the colon and 15 in the rectum. Cancer of the breast comprises 74 cases (86 samples), cancer of the prostate 26 cases (37 samples) and cancer with other localization 44 cases (47 samples). The latter group is heterogenous and consists of: bronchogenic cancer 11 cases, hypernephroma 7 cases, cancer of esophagus 5 cases, cancer of the pancreas 6 ca cancer of the bladder 4 cases and 11 cases of ot localization.

In regard to gastrointestinal cancer and m mary cancer the cases have been divided into following groups:

a) before operation without clinical signs of m stases,
b) after operation without clinical signs of rec rence or metastases and,
c) cases with proved metastases.

The latter group of gastrointestinal cancer a comprises those cases where the exploratory la rotomy showed the cancer to be inoperable. Me stases have been diagnosed clinically and by X- examinations. The cases with metastases have b divided into cases with regional and widespr metastases. As to the cancer of the breast X-ray examination has been decisive. Regard gastrointestinal cancer the division has been m difficult, because here clinical signs of even qu widespread metastases can be missing. In s cases we have employed survival time and findi at exploratory laparotomy and autopsy. Regard mammary cancer only such cases are repor where samples were taken prior to horm therapy.

As to cancer of the prostate the cases have b divided into three groups: a) without metasta b) with metastases but not treated with stilbest and c) cases with metastases treated with stilbest The diagnosis in the first group was made histological examination after transvesical enucl tion or electroresection and in the two other gro by X-ray examination of the skeleton. As a r the samples in the last group were taken 2 months after the stilbestrol therapy was induc

METHODS

The protein-bound hexose has been determi according to Björnesjö's (1955) modification of anthrone method.

Hexosamine has been determined according Blix' (1948) modification of Elson and Morga method.

otal protein in serum was determined with Van e's copper sulfate method.

ne paper electrophoresis of protein was carried n a moist chamber on Whatman No. 1 filter r. The electrophoresis was performed with nal-acetate buffer, pH 8.7, ionic strength 0.107, ent 7—8 mA, electrophoresis time 16 hours. protein strips were then stained with amido-arz 10 B according to Grassman and Hanning 1). After treatment of the strips with trans-nt oil Soalex AVL the optical density was in the photometer according to Köiv (1953) red filter and 5 mm slit. The polysaccharide s were stained according to Köiv & Grönwall 2), but instead of sulfite rinsing water 0.2 per aqueous solution of thionyl chloride was used. strips with distinct separation were photo-red; green filter and 3 mm slit were used.

STATISTICAL METHODS

tandard deviation was calculated according to ollowing formula:

$$\delta = \sqrt{\frac{d^2}{N-1}}$$

error of the difference $\varepsilon(D)$: $\varepsilon(D) = \sqrt{\varepsilon(M_1)^2 + \varepsilon(M_2)^2}$ where $\varepsilon(M)$ is $\pm \frac{\delta}{\sqrt{N}}$ for re-ive mean values. (Dahlberg 1948.)

RESULTS AND DISCUSSION

n every sample the content of hexose, osamine and protein was determined. er electrophoretic separation of protein protein-bound polysaccharides was car-out. Further, the relative polysaccharide ent was calculated as per cent of protein total and for each electrophoretic frac-The results of the polysaccharide elec-horesis are also given as relative distri-on.

he mean values of protein-bound hexose normal material given in the literature between 103—158 mg per cent, depend-ing on method of analysis (Goa, 1955). Björnesjö (1955), whose method is used in this investigation, obtained for a group of younger healthy adults a mean value of 123 mg per cent (range 106—146 mg per cent). The normal values for hexosamine show a variation between 67—97 mg per cent on determination with various modification of Elson and Morgan's method. Shetlar, Foster, Kelly & Everett (1948) have examined the variation of polysaccharides as regards age, sex and diet. They state that the content of hexose and hexosamine increases in healthy individuals with age, so that the group "aged" (61—85 years) has about 15 per cent higher content of hexose and 22 per cent higher content of hexosamine than the group of "young adults" (21—49 years). Sex and diet had no influence on hexose and hexosamine values.

On statistical treatment of the results the basic group was regarded to be that comprising operated patients, who were clinically free from postoperative metastases or recurrence. The mean values obtained for hexose and hexosamine in this group show a satisfactory agreement with normal values reported by other investigators, considering the relatively high mean age of the group and the possibility that some cases with clinically non-demonstrable metastases may be included. The difference D between the mean values for the groups without preoperative metastases, the groups with metastases, and operated patients without metastases, as well as the error of the difference $\varepsilon(D)$ are given in Table II. According to the established principles $D \geqq 3\,\varepsilon(D)$ has been considered to be statistically significant and

Table I. *Polysaccharide to protein ratio in per cent in whole serum and in electrophoretic fractions, given as mean and standard deviation, in gastrointestinal cancer, cancer of the breast, cancer of the prostate and cancer with other localizations.*

	Total	Albumin	α_1-glob.	α_2-glob.	β-glob.	γ-glob
Gastrointestinal cancer						
Without metastases, bef. operation	4.4±0.6	1.3±0.6	12.5±3.9	11.3±3.6	6.4±2.3	3.4±
Without metastases, aft. operation	3.8±0.4	1.2±0.4	9.8±4.2	9.8±2.2	6.2±1.9	2.6±
Regional metastases	4.5±0.5	1.2±0.4	13.0±6.1	13.2±2.7	6.5±2.0	3.6±
Widespread metastases	5.2±1.1	1.6±0.9	14.0±5.3	12.1±3.7	7.6±2.6	4.0±
Cancer of the breast						
Without metastases, bef. operation	3.8±0.4	1.3±0.6	9.6±3.3	9.8±3.0	5.9±1.0	3.9+
Without metastases, aft. operation	3.7±0.4	1.3±0.4	10.3±4.2	8.8±2.7	5.6±1.7	3.7±
Regional metastases	4.0±1.5	1.5±0.5	10.4±3.2	9.3±4.0	6.6±0.8	3.1±
Widespread metastases	4.7±0.5	1.8±0.5	11.5±3.4	10.9±2.5	6.1±1.7	3.7±
Cancer of the prostate						
Without metastases	4.1±0.6	2.1±0.9	9.0±3.1	9.3±1.6	5.2±1.8	2.8±
Metastases, not treated	5.1±0.8	1.6±0.7	11.7±4.2	11.6±3.6	7.9±4.6	4.4±
Metastases, treated with stilbestrol	4.0±0.4	1.2±0.7	8.9±1.6	7.9±1.9	6.4±0.9	3.3±
Cancer with other localization						
Without metastases, bef. operation	4.3±0.8	0.8±0.4	12.5±4.7	12.2±3.3	6.4±1.4	3.6±
Without metastases, aft. operation	3.5	1.0	12.6	9.6	5.2	3.1
Metastases.....	5.7±1.0	0.9±0.5	14.9±5.1	13.1±2.4	7.8±2.0	4.2±

$D \geq 2.5—3\,\varepsilon(D)$ in high degree probable. Regarding cancer of the prostate we have proceeded from the group with metastases treated with stilbestrol, the values for polysaccharides in this group showing little difference from those obtained in operated cases of mammary cancer and gastrointestinal cancer. In the heterogenous group containing cancer of other localization such a statistical evaluation could not be carried out because the number of operated cases free from metastases was not sufficient. The results of the polysaccharide determination with individual values and their means with standard deviation are presented in Figures 1, 2, 3, and 4. Statistical analysis is p sented in Table II. A statistically signific increase in protein-bound polysacchari before operation in cases without clinic demonstrable metastases was found gastrointestinal cancer (Fig. 1). Even can of other localization (Fig. 4) showed clearly elevated polysaccharide level. garding cancer of the prostate (Fig. 3) group without metastases compared to patients treated with stilbestrol showed creased carbohydrate values but without positive statistical significance. Cancer the breast (Fig. 2) holds an exceptio position, no increase of polysacchari

Table II. *Statistical evaluation of results. Difference D between the mean values for cases of manifest cancer compared to operated or to hormone-treated cases, error of the difference ε(D) and the quotient D/ε(D).*

	Without metastases, before operation			Metastases, regional			Metastases, widespread		
	D	ε(D)	D/ε(D)	D	ε(D)	D/ε(D)	D	ε(D)	D/ε(D)
trointestinal cancer									
ose	20.0	3.7	5.4	24.0	3.5	6.9	50.0	6.2	8.1
osamine	17.0	3.5	4.9	16.0	3.2	5.0	36.0	6.0	6.0
content in protein: Total	0.6	0.13	4.6	0.7	0.16	4.4	1.4	0.22	6.3
Albumin	0.1	0.13	0.8	0	0.13	0	0.4	0.18	2.2
α_1-glob.	2.8	1.07	2.5	3.2	1.88	1.7	4.2	1.28	3.3
α_2-glob.	1.5	0.79	1.9	3.4	0.87	3.9	2.3	0.82	2.8
β-glob.	0.2	0.56	0.4	0.3	0.67	0.4	1.4	0.61	2.3
γ-glob.	0.2	0.25	0.8	0	0.53	0	0.4	0.41	1.0
cer of the breast									
ose	5.0	4.0	1.2	17.0	4.6	3.7	42.0	6.6	6.4
osamine	1.0	3.6	0.3	11.0	7.5	1.5	28.0	5.4	5.2
content in protein: Total	0.1	0.11	0.9	0.3	0.21	1.4	1.0	0.14	7.1
Albumin	0	0.17	0	0.2	0.17	1.2	0.5	0.14	3.6
α_1-glob.	0.7	1.0	0.7	0.1	1.3	0.1	1.2	1.04	1.1
α_2-glob.	1.0	0.80	1.2	0.5	1.47	0.3	2.1	0.71	3.0
β-glob.	0.3	0.36	0.8	1.0	0.36	2.8	0.5	0.48	1.0
γ-glob.	0.2	0.36	0.6	0.6	0.36	1.7	0	0.30	0

cer of the prostate	Without metastases			Metastases		
	D	ε(D)	D/ε(D)	D	ε(D)	D/ε(D)
ose	15.5	7.4	2.1	49.0	7.8	6.3
osamine	8.4	7.4	1.1	34.0	6.7	5.1
content in protein: Total	0.1	0.25	0.4	1.1	0.23	4.8
Albumin	0.9	0.39	2.3	0.4	0.26	1.5
α_1-glob.	0.1	1.20	0.1	2.8	1.10	2.5
α_2-glob.	1.4	0.79	1.8	3.7	1.03	3.6
β-glob.	1.2	0.68	1.8	1.5	1.14	1.3
γ-glob.	0.5	0.37	1.3	1.1	0.59	1.9

urring in the cases free from metastases. etlar *et al.* (1949) have made the same ervation and are of the opinion that the rly normal polysaccharide values in cases skin cancer and cancer of the breast may depend on the relatively early diagnosis of these forms of cancer.

After operation of the cases without metastases and after treatment with stilbestrol of cancer of the prostate the serum

Table III. *Percentile distribution of polysaccharides in the electrophoretically separated serum fractions in cancer.*

	Albumin	α_1-glob.	α_2-glob.	β-glob.	γ-glob.
Gastrointestinal cancer					
Without metastases, bef. operation	12.3	18.4	29.9	21.5	17.9
Without metastases, after operation	15.1	14.8	26.1	21.8	22.2
Regional metastases	12.2	17.5	31.7	21.3	17.3
Widespread metastases	12.6	17.4	30.3	21.3	18.4
Cancer of the breast					
Without metastases, bef. operation	15.8	14.6	26.7	21.6	21.5
Without metastases, after operation	16.2	16.1	24.8	21.0	22.1
Regional metastases	16.3	15.4	24.7	24.8	19.0
Widespread metastases	16.2	16.8	27.4	19.6	20.3
Cancer of the prostate					
Without metastases	18.8	18.3	25.9	19.8	17.2
Metastases, not treated	12.9	15.9	32.3	19.8	19.2
Metastases, treated with stilbestrol	12.4	20.2	25.1	21.9	20.4
Cancer with other localization					
Without metastases, bef. operation	8.4	17.9	30.8	22.0	20.9
Without metastases, after operation	15.2	17.0	25.5	23.5	18.8
Metastases	6.6	20.5	35.1	21.5	16.3

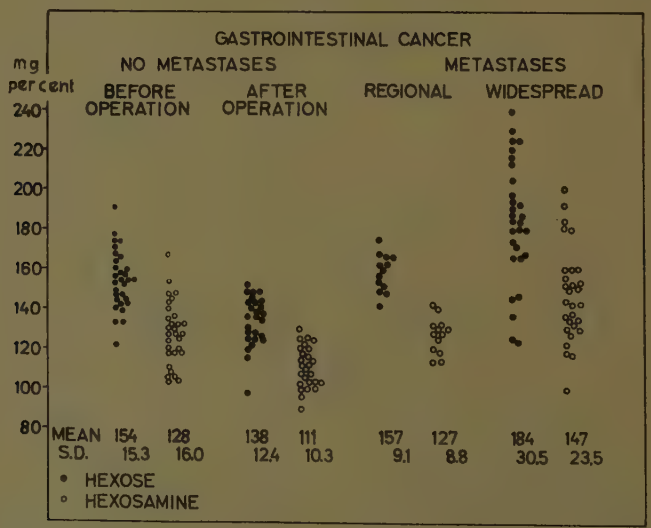


Fig. 1. Serum protein-bound hexose and hexosamine level in gastrointestinal cancer.

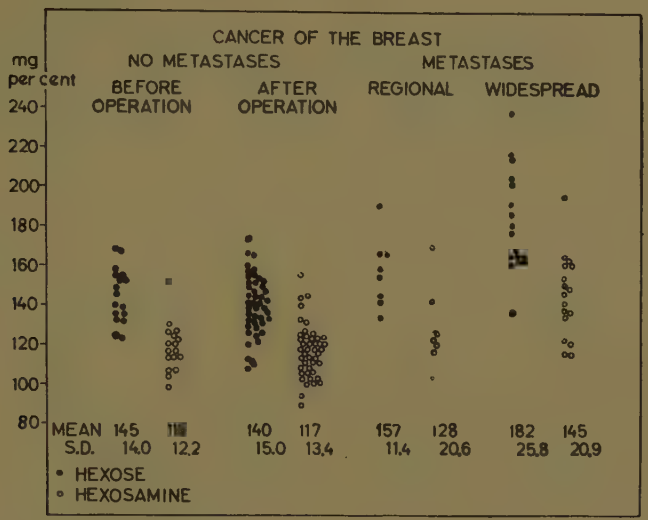


Fig. 2. Serum protein-bound hexose and hexosamine level in cancer of the breast.

saccharides showed a decrease and a ency to approach normal values.

n cases of metastases all the investigated ıs of cancer showed a statistically signifi- increase of serum polysaccharides, the ease being more marked in cases of wide- ad metastases than in those with regional astases. As to mammary cancer, how- , the increase of hexosamine became ificant first in the presence of wide- ad metastases.

he relative content of polysaccharides in ein is shown in Table I. It showed an ease in those groups where the absolute ent of hexose and hexosamine were in- sed. The quotient was clearly higher re operation in gastrointestinal cancer, not in mammary cancer or cancer of prostate without metastases. In the pre- e of metastases the quotient was signi- ıtly increased in all the groups.

Whether there is a general increase in polysaccharide content of the serum protein or whether one or more special components rich in polysaccharides occur in an unusually high concentration, is a problem that can only be solved by examining the relative content of the polysaccharides in serum protein fractions. A tendency toward increased content of polysaccharides in the albumin fraction was found in cases of widespread metastases. This increase was considerable in mammary cancer, but even in cancer of the prostate and cancer of the gastrointestinal tract, the rise was evident. The increase, however, was not so high as earlier reported by Shetlar *et al.* (1950). The content of polysaccharides in α_1-globulin was considerably increased in gastrointestinal cancer in cases without preoperative metastases as well as in those with metastases. For mammary cancer

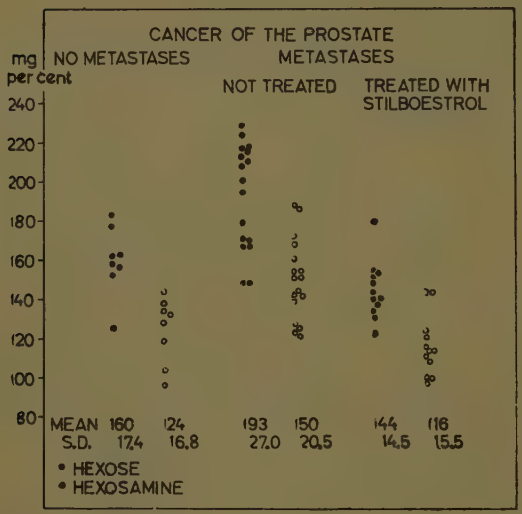


Fig. 3. Serum protein-bound hexose and hexosamine level in cancer of the prostate.

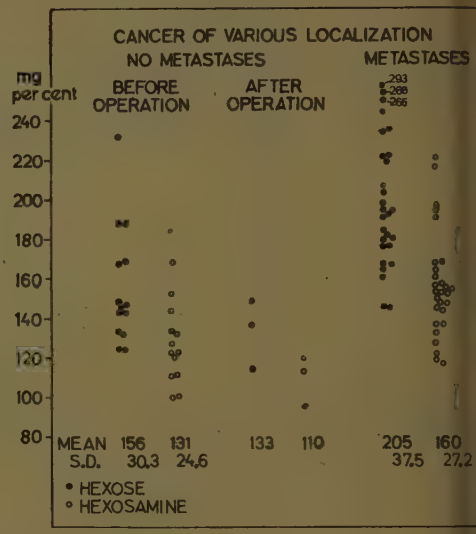
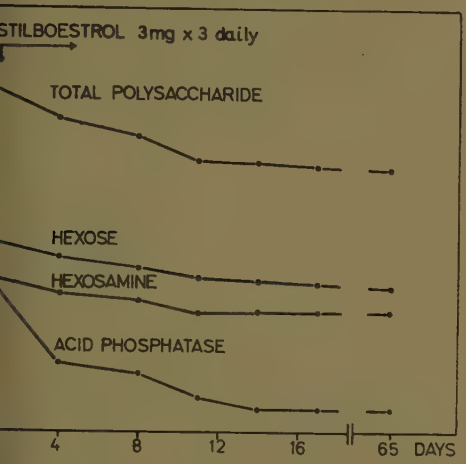


Fig. 4. Serum protein-bound hexose and hex amine level in cancer of various localizations.

no statistically certain difference in the various groups was found. For cancer of the prostate an increase was noted in cases with metastases. As to α_2-globulin it was observed that the relative content of the polysaccharides showed a tendency to increase in cases without preoperative metastases in gastrointestinal cancer. In cases with metastases this increase was statistically significant for gastrointestinal cancer and cancer of the prostate. The increase was less clear in the cases of mammary cancer with metastases, where it became probably significant first in cases of widespread metastases. The relative content of polysaccharides in β- and γ-globulin was on the whole the same in cases without preoperative metastases in comparison to the operated cases. In cases with metastases the quotient for β-globulin showed a tendency to increase. This tendency, however, had no cert statistical significance. The content of p saccharides in γ-globulin was mainly c stant irrespective of clinical change. mentioned earlier, no statistical evaluatio the results for the heterogenous group cancer with other localization was ma The changes in the relative content of p saccharide in the protein fractions in group seemed to follow those stated cancer in the alimentary canal, α_1- and globulin being most rich in polysacchari

The relative distribution of polysacc rides in the electrophoretically separa fractions is shown in Table III. The grea changes occurred in the α_1- and α_2-fracti especially in the groups with metasta where increased values were found.

Fig. 5 demonstrates the changes in content of hexose, hexosamine and

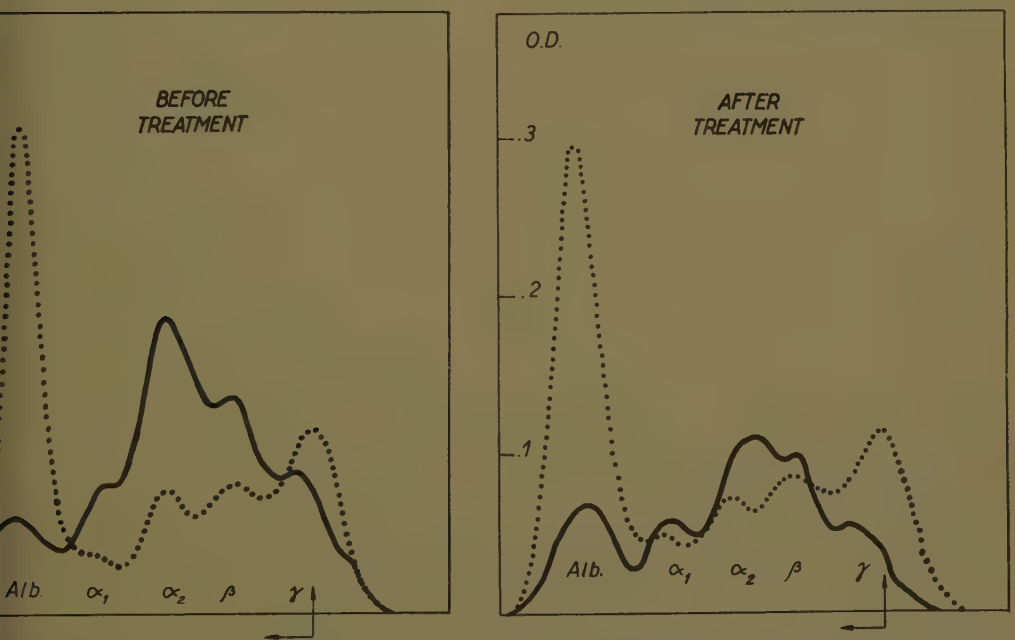


5. Changes in hexose, hexosamine and acid hatase level in serum after administration of trol in a case of cancer of the prostate with skeletal metastases.

phosphatases in serum for a case of cancer of the prostate with skeletal metastases under treatment with stilbestrol. The content of the polysaccharides decreased and reached a constant level after 17 days treatment. Moreover, it was found that this decrease paralleled that of the serum acid phosphatases.

Fig. 6 shows the relative distribution of proteins and polysaccharides for the same case of cancer of the prostate with metastases before treatment and after 14 days treatment with stilbestrol.

We cannot, for the time being, make recommendations concerning the clinical use of the above results, but it is probable that the determination of serum polysaccharides can be of use in investigating malignant disease, if other pathological conditions with in-



Electrophoretic patterns of serum proteins (dotted line) and polysaccharides (solid line) in a canser of the prostate with skeletal metastases before and after 14 days treatment with stilbestrol.

creased polysaccharides can be excluded. Further, the observations concerning cancer of the breast, where polysaccharides became increased first after the occurrence of metastases, are perhaps of a certain clinical importance. We assume that the observations concerning the increase in polysaccharides in connection with dissemination of the cancer ought to be made the object of further clinical investigations. As changes in protein-bound hexose and hexosamine occur in parallel, the determination of the content of hexose, being a simpler laboratory procedure, would be clinically sufficient.

SUMMARY

224 cancer patients were examined regarding the content of the protein-bound serum polysaccharides and their distribution in the electrophoretic fractions. The localization of the cancer and the clinical picture were used as the basis for grouping the cases. A preoperative increase of hexose and hexosamine in cases free from metastases has been found in gastrointestinal cancer, cancer of the prostate and in cancer of other localization, with the exception of cancer of the breast, where no increase occurred. In case of inoperability or after the occurrence of metastases the high level of the polysaccharides was even more marked and was significant also in case of mammary cancer. By paper electrophoretic separation of protein and polysaccharides it was possible to establish that the relative content of the polysaccharides increased most in α_1- and α_2-globulin. Changes of the relative content of polysaccharides in the albumin and β-globulin fractions were observed in cases with metastases, but they lack definitive statistical significance.

ACKNOWLEDGEMENT

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SPECTROPHOTOMETRIC DETERMINATION OF HEMOGLOBIN OXYGEN SATURATION IN HEMOLYZED WHOLE BLOOD BY MEANS OF VARIOUS WAVELENGTH COMBINATIONS

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A number of different wavelength combinations have been employed in the spectrophotometric methods for determination of hemoglobin oxygen saturation in blood. The present investigation has been undertaken in order to determine the accuracy of a method, when three different wavelength combinations are employed, and further to determine the effect of increased content of bilirubin and lipids in the blood, and of smoking.

Optical density was read successively at three characteristic and frequently employed wavelengths, 576 and 560 mμ, a maximum and a minimum in the absorbtion curve of oxyhemoglobin, and 506 mμ, an isobestic point for the absorption curve of oxyhemoglobin and hemoglobin, and the oxygen saturation was calculated with the combinations 576—560, 576—506 and 560—506.

The combination 576—560 has been used by Klungsøyr & Støa (1954) (575—558) and Refsum & Sveinsson (1956), and 576—506 by Tsao, Sethna, Sloan & Wyngarden (1955), while results with the combination 560—506 have previously not been published.

METHODS

Procedure. Immediately after hemolysis the optical densities were read at 20° C on a Beckman Spectrophotometer Model DU in the sequence 576, 560 and 506 mμ (slit 0.04), using Klungsøyr & Støa's ultramicrocuvette. For details see Klungsøyr & Støa (1954), Refsum & Sveinsson (1956) and Refsum (1957).

Calculations. The hemoglobin oxygen saturation was calculated according to the formula for a spectrophotometric two-component system (Drabkin 1951):

$$\text{Percentage } HbO_2 = 100 \cdot \frac{\frac{D\lambda_1}{D\lambda_2} \cdot \frac{DHb\lambda_2}{DHbO_2\lambda_2} - \frac{DHb\lambda_1}{DHbO_2\lambda_2}}{\frac{D\lambda_1}{D\lambda_2}\left(\frac{DHb\lambda_2}{DHbO_2\lambda_2} - 1\right) + \frac{DHbO_2\lambda_1}{DHbO_2\lambda_2} - \frac{DHb\lambda_1}{DHbO_2\lambda_2}}$$

λ_1 and λ_2 being the wavelengths employed, D the optical densities of the sample at these wavelengths, $DHb\lambda_{1\text{-}2}$ and $DHbO_2\lambda_{1\text{-}2}$ the optical densities of blood with 0 and 100 per cent oxygen saturation respectively. The quotients in which $DHb_{1\text{-}2}$ and $DHbO_2\lambda_{1\text{-}2}$ are concerned, are constants which need be determined only once. The calibration curve $D\lambda_1/D\lambda_2$ *versus* percentage Hb is slightly curved. When λ_2 represents an isobestic point (*e. g.* 506 mμ), $DHbO_2\lambda_2 = DHb\lambda_2$, the formula is simplified and the calibration curve linear.

RESULTS AND DISCUSSION

Spectrophotometric constants for the calculation of hemoglobin oxygen saturation were determined in 10 different blood samples (Table I).

The oxygen saturation was determined spectrophotometrically by means of the th

elength combinations and manometrically
n Slyke, 1924) in 23 different blood
ples (Table II).

he mean difference between the duplicate
ings was 0.2 for 576—560, 0.7 for 576—
and 0.6 for 560—506, while the corre-
ding standard deviations were 0.15, 0.43
0.44 respectively.

he results show that in the determination
he spectrophotometric constants as well
n the analyses, the best agreement be-
en duplicate readings was found with the
elength combination 576—560, where the
erence between $DHbO_2\lambda_1/DHbO_2\lambda_2$ and
$bλ_1/DHbλ_2$ is large, while at the same
the absolute values of these quotients
small. Further it is seen that the agree-
t between the spectrophotometric and
ometric results is somewhat better with
combination 576—560 than with any of
other two combinations.

he greater degree of inaccuracy, both in
determination of the constants and in the
yses at the wavelength combinations
—506 and 560—506, is related to the
culty of obtaining readings which are
esentative for the isobestic point (506
). A slight shift of the isobestic point or
ight error in the reading leads to a con-
rable error in the calculated quotient,
cially with the combination 576—506.
the other hand, D506 remains constant
r hemolysis, while D576 and D560 often
nge rather quickly, due to a reduction
he oxygen saturation (Refsum, 1957).
he procedure for the determination of
of the constants for the wavelength
bination 576—560 is technically some-
t complicated, because both $DHbO_2$ and
b must be determined in the same blood

Table I. *Spectrophotometric constants for calculating hemoglobin oxygen saturation with the wavelengths 576—560, 576—506 and 560—506 mμ, determined in 10 different blood samples at 20° C.*

	Mean	S. D.
$DHbO_2576/DHbO_2560$	1.807	0.002
$DHb576/DHbO_2560$	1.094	0.005
$DHb560/DHbO_2560$	1.470	0.005
$DHbO_2576/DHbO_2506$	3.161	0.006
$DHb576/DHb506$	1.915	0.007
$DHbO_2560/DHbO_2506$	1.750	0.004
$DHb560/DHb506$	2.570	0.009

Table II. *The hemoglobin oxygen saturation in per cent, determined spectrophotometrically in hemolyzed whole blood with the wavelength combinations 576—560, 576—506 and 560—506 mμ, and manometrically.*

Spectrophotometry			Manometry
576—560	576—506	560—506	
9.2	8.2	10.4	9.3
13.4	13.9	12.6	13.8
18.1	17.7	18.9	18.4
20.1	19.7	20.7	20.3
22.0	22.8	21.8	23.2
34.1	33.9	34.0	34.7
47.3	48.5	45.7	47.5
52.2	52.0	53.0	54.0
58.2	59.2	56.6	57.9
71.8	72.4	70.8	72.4
83.0	81.7	83.9	83.6
83.7	84.0	83.1	84.4
84.3	84.5	84.1	82.6
85.2	84.6	85.8	87.5
87.4	85.6	88.9	87.2
91.1	90.4	91.8	92.2
92.4	90.6	93.9	92.6
92.9	93.2	92.7	90.8
93.8	93.8	93.8	93.7
94.0	92.4	95.2	93.1
94.0	94.7	93.3	95.0
94.6	94.8	94.5	93.0
95.9	95.1	96.2	96.9

sample at exactly the *same* depth of cuvette and at the *same* concentration of total pigment. (See the calculation formulas.)

Tsao, Sethna, Sloan & Wyngarden (1955), among others, found sodium dithionite unsatisfactory as a reducing agent in determination of the spectrophotometric constants where DHb is involved. Table II shows that there is good agreement between the spectrophotometric and manometric results also at low oxygen saturations. This indicates that the procedure employed for reduction (6 mg $Na_2S_2O_4$ per ml hemolyzed blood) is adequate for determining constants for use in determination of oxygen saturation in the range investigated. In some additional investigations the same values were obtained for DHb regardless of whether the calculated quantity of sodium dithionite, or two or three times as much, was used, and the values remained unchanged for more than one half hour.

Analytical errors due to inaccurate adjustment of temperature are dependent both on the wavelength combination and the oxygen saturation, but are greatest at the combination 576—506 and usually smallest at 560—506 (Refsum, 1957).

The effect of increased content of bilirubin and lipids in plasma, and the influence of smoking

The optical densities were determined in plasma samples with varying content of bilirubin. While D576 and D560 were practically zero at bilirubin contents up to 20 mg per cent, D506 increased considerably. Hence the determination of oxygen saturation with the wavelength combination 576—560 was not influenced even by high bilirubin content. But with the combination 576—506 in tv fully oxygenated blood samples with bilir bin contents of 19.7 and 3.4 mg per ce (with normal hemoglobin concentration a hematocrit) the oxygen saturations obtaine were too low, respectively 88.3 and 97.7 p cent. Correspondingly, with the combinatic 560—506, the values were too high, respect vely 109.4 and 101.7 per cent. At decreasin levels of oxygen saturation the error w increase for the combination 560—506 a decrease for 576—506.

The optical densities were similarly dete mined after a meal with high fat conte (cream), and an increase was found at a wavelengths, highest at 506, lowest at 57 mμ. Fully oxygenated blood samples from healthy person with normal hemoglobin a hematocrit, and fasting plasma lipids of 73 mg per cent were investigated. After increas of the plasma lipids to 910 and 1050 m per cent, the oxygen saturations found we respectively 99.7 and 99.3 per cent at th wavelength combination 576—560, 98.0 a 95.3 per cent at 576—506 and 101.4 a 102.8 per cent at 560—506. At lower satur tions the error at 576—560 and 576—5 will be somewhat smaller, at 560—506 som what greater.

Analytical errors due to a particular co tent of bilirubin or lipids in the plasma w be greater at low hemoglobin and hematoc values, and will be more than doubled wh the hemoglobin concentration decreases half.

The analytical errors due to increas bilirubin or lipid content can be eliminat by subtracting optical density for plasma (1—hematocrit) from the optical density f the hemolyzed whole blood at the respecti

elengths, and otherwise using the usual trophotometric constants in calculating oxygen saturations. Alternatively, one use the uncorrected optical densities for hemolyzed blood and make a special rmination of the spectrophotometric con- s in the same blood.

lood samples of 5 ml were taken from a ker (5—10 cigarettes per day) *before after* smoking two cigarettes. After equi- tion with 600 ml pure oxygen for about minutes, the oxygen saturations at the elength combinations 576—560, 576— and 560—506 were 99.1, 99.0 and 99.2 cent in the sample *before* smoking, and 95.9 and 96.6 per cent in the sample . Analytical errors due to the presence COHb and other inactive hemoglobin vates may be corrected by inserting con- s in the formula for calculating oxygen, rmined especially for the blood sample uestion.

n the basis of these findings the combina- 576—560 is recommended.

SUMMARY

ne hemoglobin oxygen saturation has determined spectrophotometrically in hemolyzed whole blood in Klungsøyr & Støa's ultramicrocuvette with the wavelength combinations 576—560, 576—506 and 560—506 mμ. On comparison of the analytical results, and investigation of the influence of increased content of bilirubin and lipids in the plasma, as well as increased content of COHb in the blood of smokers, the combination 576—560 mμ is found to be the most advantageous.

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DOES HUMAN BLOOD CONTAIN URIC ACID RIBOSIDE

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In 1922 uric acid riboside was isolated from oxblood by Davis, Newton & Benedict and identified by elementary analysis and by means of the acid hydrolysis products of the compound, *viz.* uric acid and d-ribose. Later the presence of uric acid riboside in ox-blood was verified by Falconer & Gulland (1939), who further showed that the compound was uric acid-9-d-riboside.

Moreover, Newton & Davis (1922) produced evidence for the presence of uric acid riboside in human blood and, in traces, in the blood from horse, dog, pig, chicken and sheep. Since the chemical basis of these findings is not too well established, the question of the presence of uric acid riboside in human blood has occasionally been raised in the literature (Kalckar, 1947; Van Slyke, 1946; Jørgensen & Theil Nielsen, 1956).

The present investigation was carried out to contribute to the final clarification of this problem, first by determining the optimal conditions under which the uric acid riboside in deproteinized ox-blood is hydrolyzed and, secondly, by determining if any uric acid is released when deproteinized human blood is subjected to the same hydrolysis conditions.

METHODS

Analytical method

The determination of uric acid was carried out by enzymatic differential spectrophotometry according to the method described by Prætorius & Poulsen (1953).

Experimental procedure.

Hydrolysis of uric acid riboside in deproteinized ox blood:

Fresh, heparinized ox-blood was deproteiniz by pouring 100 ml blood, while stirring, slow into 5 volumes of boiling 0.01 M-acetic acid. Af 2 minutes of boiling the denatured proteins w filtered off, when still warm, on a big foldi filter.

The complete clear, yellow filtrate was evapo ted *in vacuo* at 50 °, to the original blood volu This filtrate was used for hydrolysis experime

The filtrate was acidified with 2.5 M-H_2S to give a final concentration of 0.25 moles per li and 1.0 ml aliquots were placed in ampules a heated after sealing in a glycerin bath at 98 ° at 125 °.

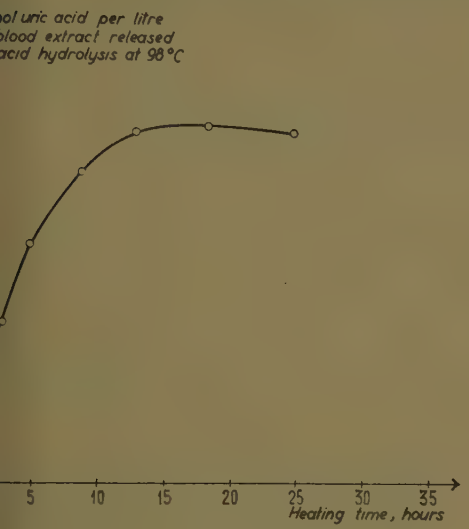
At various specified time intervals ampules w removed and cooled. The content of the ampu was neutralized and buffered with 0.6 M-glyc pH 9.2 and analyzed for uric acid.

Experiments with deproteinized human blo

5 ml samples of fresh, heparinized venous bl were poured, while stirring, into 25 ml of boil 0.01 M-acetic acid. The precipitated protein filtered off and the clear filtrate was evapora to dryness at 50 ° *in vacuo*. The residue was solved in 4.0 ml 0.03 M-glycine buffer pH 9.2, 50 μl uricase was added to remove the free acid present. Uric acid riboside is not attacked this enzyme. It was checked after one hour no free uric acid was present.

2 ml of the reaction mixture and 0.2 ml 5

[1] This investigation was aided by grants f "Kong Christian den Tiendes Fond" and "Sta alm. Videnskabsfond".



1. Release of uric acid produced by acid hydro- of ox-blood containing uric acid riboside. Temperature 98° C.

O_4 were placed into an ampule and heated for ours at 125 °. After cooling the content of the ule was neutralized, buffered with 0.6 M-glycine 9.2, and analyzed for uric acid.

RESULTS

igure 1 shows that the release of uric acid eproteinized ox-blood during hydrolysis .5 N-H_2SO_4 at 98 ° comes to an end at -13 hours. The liberated amount of uric corresponds to an initial concentration ric acid riboside of 1.08 mM per litre of lood extract.

n hydrolysis experiments performed at ° a total hydrolysis was obtained after hours. Prolonged heating at this tempe- re seemed to be accompanied by a slow mposition of uric acid. After heating for 3 and 4.5 hours at 125 °, the concentra- of uric acid was 1.11, 1.06 and 1.00 mM litre ox-blood extract, respectively.

fter hydrolysis of blood from four dif- ferent persons no release of uric acid could be measured. Considering the limitations of the method used, it can be argued that less than 0.0015 mM uric acid per litre blood is present in combined form.

These results indicate that if any uric acid riboside is present at all in human blood, the amount is so small that it cannot be demonstrated with any significance by means of the present highly specific and sensitive method.

SUMMARY

A quantitative hydrolysis of uric acid riboside in deproteinized ox-blood is obtained in 0.5 N-H_2SO_4 after 12—13 hours at 98 ° and after 1.5 hours at 125 °.

Under this treatment deproteinized human blood give off no measurable amount of uric acid. This was substantiated by means of a highly specific and sensitive method for the determination of uric acid.

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DETECTION AND ROUGH ESTIMATION OF HOMOGENTISIC ACID IN URINE

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(Received for publication July 7, 1956)

The presence of homogentisic acid or "alkapton" (Bödeker, 1859) in urine is primarily recognised by the dark color of the urine, providing it is alkaline. However, more spesific methods are obviously needed, and a series of procedures have been recommended. One of the simplest and most specific methods is that devised by Fishberg (1942). It is based upon the reducing capacity of the hydroquinone groups of homogentisic acid acting as a developer on a photographic film.

The procedure is described as follows: "The urine is made strongly alkaline with sodium or potassium hydroxide and dropped on regularly sensitized photographic paper. Wherever a drop of the urine touches the paper, the latter turns coal black instantly, ——. This may be done in full daylight." Fishberg states that no other urinary substance is capable of reducing photographic paper in this way and that an alkaptonuric urine will always give a positive reaction.

During our experiments on an alkaptonuric patient, we found that his urine sometimes gave a very faint "Fishbergs reaction" and that normal urine regularly produced a faint spot on the paper. This initiated further investigations concerning the method and a somewhat altered procedure is recommended.

METHOD

Hydroquinone standard.

0.262 g of crystalline hydroquinone is dissolved in 50 ml of normal urine. The solution is protected from light and renewed every day.

Developer standard.

Kodak D19 b is dissolved in water as instruct for ordinary use, and then diluted 1 : 2 with wat

Procedure.

An ECG-film strip is exposed to daylight f at least 10 minutes. 0.5 ml of a 10 per cent sodiu hydroxide solution is added to 2 ml of urine a test tube. Within 10 minutes a drop of the alkali urine is added to the film in the dark room. T strip is rinsed with water after 6 minutes a fixed in an ordinary bath for 10—15 minutes. T appearance of a grey to black spot indicates t presence of homogentisic acid in the urine samp

In order to get a rough estimation of the hom gentisic acid concentration, the darkness of t spot, if necessary after appropriate urine dilutio may be compared with a standard scale of spo This is obtained by preparing a geometrical dil tion series of the hydroquinone or the develop standard solution and treating each tube in t series as described above for the urine. The u diluted standards are equal to a homogentisic ac concentration of 0.80 per cent. The third spot generally a little weaker than the first two, a the seventh spot (dilution 1 : 64) hardly visi (Table VII).

RESULTS AND DISCUSSION

Influence of daylight

When the reaction was performed in da light, definite although faint spots for t normal controls were regularly obtained, contrast with the statement of Fishberg. Th was avoided if the reaction took place in t dark room.

Fixation

It is necessary to fix the film after a r action performed in the dark room. In t "daylight procedure" such fixation is u necessary, but if it is performed, all the spo including the normals, will appear mo distinct.

[1] With the assistance of Miss B. L. Stewart, M. Sc., New Zealand.

le I. *Influence of amount of sodium hydroxide on the darkness of the spots. +++ indicates a coal black spot, (+) a spot which is hardly visible.)*

10 per cent NaOH	Urine containing 0.89 per cent homogentisic acid		Urine containing 0.22 per cent homogentisic acid	
ml	pH	Darkness of the spots	pH	Darkness of the spots
0.03	6.8	0	11.1	+
0.10	9.4	+	12.1	++++
0.15	10.3	+++(+)	12.0	++++
0.20	11.4	++++(+)		
0.30	11.9	+++++		
0.50	11.8	+++++		

Length of light exposure

he film was exposed to light for a short l of time (*e. g.* 15 seconds) before the on, the resulting spots were very pale accordance with ordinary photographic omena. Maximum darkness of the spots obtained with a longer light exposure varying from one to five minutes with ent type and strength of light. If a mini- exposure time of 10 minutes is applied, timal result should always be obtained the present type of film.

mount of sodium hydroxide added

addition of an insufficient amount of to the urine, the resulting spots were or invisible. The pH had to be raised out 12 to give maximum darkness of ot (Table I). This was usually obtained e addition of 0.5 ml of a 10 per cent m hydroxide solution (8—10 drops) ml of urine.

Elapse of time after alkali addition

spot tests were performed at intervals the addition of the appropriate amount dium hydroxide, the darkness of the ing spots decreased (Table II). This de- began earlier if the homogentisic acid nt of the urine was low (Table III). It ecognized after 10—15 minutes, and con- l until spots were no longer produced 2—16 hours). This happened although the pH of the mixture remained above the critical level. Further addition of sodium hydroxide did not increase the density of the spots. With an initial insufficient addition of sodium hydroxide, only 4 weak spots developed, and as before a further decrease was found on repeated tests. At the same time there was a decrease in pH. If this was raised by another addition of sodium hydroxide, a darker spot developed, which shows that the homogentisic acid had not been completely destroyed. Hence the decrease in darkness in this instance is due partly to a destruction of homogentisic acid and partly to a decrease in pH.

"Developing time"

If drops of alkaline urine were allowed to act on the film for a short period of time, the resulting spots were pale. With increasing time of contact the spots were darker and maximum response was obtained after about 6 minutes contact (Table IV).

This was true also for other concentrations of homogentisic acid.

Urinary concentration of homogentisic acid.

According to Fishberg "the reaction is unlikely to be negative in an alkaptonuric patient at any time". However, under certain conditions the urinary excretion of homogen-

Table II. *Influence of time elapsed after addition of sodium hydroxide on the dar ness of the spots. The urine contains 0.89 per cent homogentisic acid.* (+ + + + + *a coal black spot,* (+) : *hardly visible.)*

Amount of 10 per cent NaOH	9 minutes		21 minutes		39 minutes		60 minutes		70 minutes Extra additio of 0.20 ml o 10 per cent Na	
ml	pH	Spot	pH	Spot	pH	Spot	pH	Spot	pH	Spot
0.03	6.8	0	6.9	0	7.0	0	6.9	0	11.35	+++
0.10	9.4	+	9.25	+	9.15	+	9.0	(+)	11.65	++++
0.15	10.3	+++(+)	10.2	++	10.0	+(+)			11.8	++++
0.20	11.3	++++(+)	11.0	+++	10.8	++			11.8	++++
0.30	11.9	+++++	11.9	+++++	11.7	++++	11.4	++	11.8	+++
0.50	11.8	+++++	11.85	+++++	11.8	++++(+)	11.85	+++(+)	11.7	++(+)

Table III. *Influence of time elapsed after addition of sodium hydroxide on the dar ness of the spots. The urine contains 0.22 per cent homogentisic acid.* (+ + + + + *a coal black spot,* (+) : *hardly visible.*

Amount of 10 per cent NaOH	3 minutes		16 minutes		30 minutes		60 minutes Extra addition of 0.20 ml of 10 per cent NaO	
ml	pH	Spot	pH	Spot	pH	Spot	pH	Spot
0.06	11.45	+(+)	11.15	(+)	10.75	(+)	11.95	++
0.10	12.1	++++	12.05	+++	12.0	++	11.8	+
0.15	12.0	++++	12.0	+++	12.05	++	11.85	+

tisic acid may diminish, and especially if this coincides with a large diuresis the concentration may be low enough to result in a gray spot (Table V). A certain correlation can be recognised between the darkness of the spots and the homogentisic acid concentration, as determined by the method of Metz (1927), modified by Lieb & Lanyar (1927) and by Metz (1930). This was also found for dilution series of alkaptonuric urines (Table VI).

With a suitable standard scale of spot is thus possible to estimate roughly the c centration of homogentisic acid in the sam

As homogentisic acid is not easily availa we made some experiments with hyd quinone as standard. If the hydroquin group in both reagents has the same de oping (reducing) ability, 1 g of homogent acid should equal 0.655 g of hydroquin This was in fact the case, as shown

le IV. *Influence of time of contact be- the drop and the film on the darkness spots. The urine contains 0.16 per cent gentisic acid. (+++++: a coal black spot, (+): hardly visible.)*

linutes of contact	Darkness of the spots
1	+(+)
2	++
4	++(+)
6	+++(+)
8	+++(+)
10	+++(+)

Table V. *Variations in the homogentisic acid concentratoin in the urine during one day and the darkness of the corresponding spots. (+++++: a coal black spot, (+): hardly visible.) The patient took a low protein diet with an addition of 3 g tyrosine orally at 6 a. m.)*

Urine sample collected at	Homogentisic acid concentration in per cent	Darkness of the spots
6 a.m.	0.33	++++(+)
8 a.m.	0.89	+++++
10 a.m.	1.23	+++++
12°°	1.35	+++++
2 p.m.	0.61	+++++
4 p.m.	0.19	++++
6 p.m.	0.09	+++

ble VI. *Spots resulting from geometrical dilution series of an alkaptonuric urine and an equimolecular solution of hydroquinone in normal urine, in water and some salt solutions. (+++++: a coal black spot, (+): hardly visible.)*

tion	Homogentisic acid concentration in per cent	Darkness of the spots	Hydroquinone concentration in per cent	Darkness of the spots with normal urine as solvent	Darkness of the spots with water (or salt solutions) solvent
1	0.89	+++++	0.58	+++++	+++++
2	0.45	+++++	0.29	+++++	+++++
4	0.22	++++	0.15	++++(+)	+(+)
8	0.11	+++(+)	0.07	+++(+)	(+)
16	0.06	+++	0.036	+++	(+)
32	0.03	+(+)	0.018	++	(+)
64	0.015	(+)	0.009	+	0
128	0.0075	0	0.0045	0	0

e VI, where the hydroquinone concen- on is chosen to correspond to the homo- sic acid concentration in the original . To find a more suitable solvent for the oquinone standard than the normal urine oyed in Table VI, we tried different solutions ($MgSO_4$, NaCl, $NaHSO_3$, in commercial developer) without suc- They all gave the same results as plain r: an abrupt fall in spot strength (Table last column) completely different from low decrease seen for homogentisic acid for hydroquinone in urine.

A commercial developer for films (Kodak D19 b) was tried in half the ordinary concentration, and it gave a result more similar to the urines (Table VII).

The best standard is apparently hydroquinone dissolved in normal urine.

SUMMARY

A modified film spot test (Fishberg) for homogentisic acid is described and a standardised procedure recommended. A rough

Table VII. *Standard scales of spots resulting from geometrical dilution series of hydroquinone in urine and of a commercial developer.* (+ + + + + : *a coal black spot,* (+) *hardly visible.)*

			Darkness of the spots			
Dilution	Hydroquinone concentration in per cent	Corresponding homogentisic acid concentration in per cent	Hydroquinone in normal urine	Developer ½ ordinary strength in water	Developer minus salt portion in water	Developer minus salt portion in normal urin
1:1	0.52	0.8	+ + + + +	+ + + + +	+ + + + +	+ + + + +
1:2	0.26	0.4	+ + + + +	+ + + + +	+ + + + (+)	+ + + + +
1:4	0.13	0.2	+ + + +	+ + + + (+)	+ + + +	+ + + + +
1:8	0.065	0.1	+ + +	+ + + (+)	+ + + (+)	+ + + +
1:16	0.033	0.05	+ +	+ +	+ (+)	+ + +
1:32	0.016	0.025	+	(+)	(+)	+
1:64	0.008	0.0125	0	0	0	0

estimation of the homogentisic acid concentration in the alkaptonuric urine can be obtained by this very simple method.

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A SUCTION DEVICE FOR PIPETTES

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lling of pipettes by oral suction may nt inconveniences and even be danger- in case of poisonous, corroding of in- us fluids.

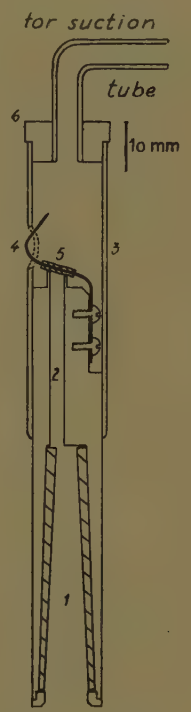
overcome these hazards several ingeni- types of pipette suction devices have constructed. A major inconvenience is the tip of the pipette is impossible to ol accurately using one hand only, as derable force is usually necessary.

e following construction functions in ection with a water suction-pump, and sily operated by small pressures of the b.

few explanations will be necessary (see . The conical receiver (lower end) will t all commonly used pipettes tightly and ely because of the soft replaceable poly- chloride gasket. This is made from a with 4 mm lumen and 2 mm wall. A al shape is obtained by pulling the tube a metal cone with an angle a little r than desired for the finished product. and tube are warmed in glycerol to C, then cooled in tap water. The canal the receiver is continued through a obliquely cut tubelike extension of the al metal core. Airtight closure is here ted by an S-curved and tempered steel g, 5 mm wide and 0.5 mm thick, and l with a short length of rubber tube.

e fit between the outer metal cylinder er part) and inner cylindrical core d be practically airtight.

e suction device and pipette is prefer- gripped like a dagger, thus supporting



Axial section through suction device for pipettes.

1. Conical receiver with plastic gasket (hatched). 2. Eccentric canal in central metal core. 3. Outer metal cylinder, tightly fitting over central core. 4. Tempered steel spring emerging though hole in outer metal cylinder and fixed in central metal core by two screws. 5. Short piece of rubber tubing permitting air-tight closure over obliquely cut tubelike extension of central core. 6. Soldered metal cover on outer cylinder, carrying a metal tube for connection with suction.

the pipette with the proximal part of the hypothenar.

The bent metal tube is connected to a water suction-pump in moderate action. No suction is exerted on a pipette inserted in the receiver as air is drawn through the opening in the outer metal cylinder and the canal to the receiver is closed by the spring. Suction is started by letting the pulpa of the thumb close the hole in the cylinder and at the same time pressing on the bend of the spring. As no vacuum has been built up previously, the initial suction is gentle, but rapidly becomes maximal. When the solution approaches the mark on the pipette, the pressure of the thumb is gradually relased and ceases at the appropriate moment. The exact adjustment will be rapidly achieved with short practice.

Should the volume mark be overreached, the adjustment is effected by a slight pressure on the spring with the tip of the thumb and without closing the opening in t cylinder. In the same way the vacuum the pipette is relieved when the pipette is be emptied.

With a spring of appropriate streng the force necessary for manipulations slight.

It should be noted that when neith spring nor hole is touched, the tip of empty pipette may be inserted in the soluti without being filled, and a full pipette c be removed from the solution in the sa way.

The pipette is fully controlled all the tim thus the tip and the exact adjustment of t meniscus may be conveniently observed.

ACKNOWLEDGEMENT

Mr. Holger Fallesen, chief mechanic at the I stitute for Physical Chemistry, University Copenhagen, is gratefully thanked for valua technical suggestions and excellent workmansh

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A SIMPLE TYPE OF BURETTE WITH AUTOMATIC ZERO ADJUSTMENT

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he principle of self-adjustment for ettes is valuable and has been solved everal ways (*i. a.* Daffert, Derona and wczinski types).

ll types, however, are intricate and conse-ntly expensive and vulnerable. Cleaning xtremely difficult especially with deposits he tips at the zero point and breakage is frequent.

he following simple burette has been le (Fig. 1, 2) and found to present some antages.

thick walled capillary tube 50 mm long a bore of about 0.5 mm and 6—7 mm rnal diameter is fused on the "zero end" good, graded burette tube so that the lting zero lies at the free end of the llary. This is finely ground to a conical The whole of the capillary only contains l, thus corresponding to one small divi- on the usual one- or two-milliliter ettes.

he other end of the burette tube is fused the single tube of a two-way stopcock two oblique bores. In burettes for 1—5 a capillary-tubed stopcock is used, for ater volumes thick tubes are better. One he other two tubes (*i. e.* the right as one facing the burette) is drawn to a point, other bent to the side to receive the ng from the reservoir, which is either ed high or fitted with double bored stop- and rubber bulb. Other types of two-way cocks and arrangement of the connec- s can of course be used, *i. a.* a stopcock Y-bore and afferent tube on the upper side (as in most Derona burettes). Also the afferent glass tube may be fitted in the stopper of a reservoir bottle and extended to the bottom; filling is then effected by pressure.

The upper capillary end of the burette is fitted with a simple housing for overflow, *i. e.* a glass tube about 30 mm in diameter,

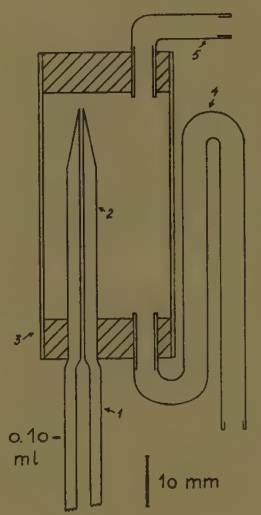

Fig. 1. Axial Section through Upper End of Burette with Automatic Zero Adjustment and Overflow-chamber. 1. Upper end of graded burette glass tube. 2. Thickwalled capillary tube fused on burette tube and ground to a conical tip. 3. Overflow-chamber made of glass tubing and closed by rubber stoppers. 4. S-shaped glass tube for emptying of chamber and ensuring against backflow. 5. Ordinarily the chamber is emptied by a puff in the short glass tube.

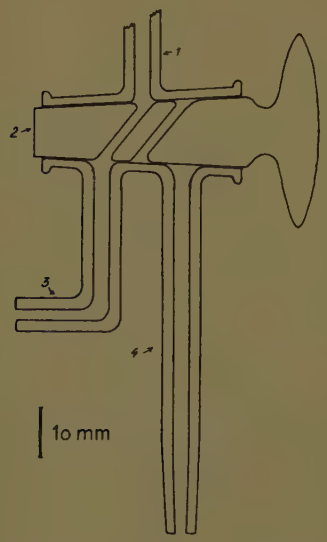

Fig. 2. Axial Section through Lower End of Burette. 1. Lower end of burette tube (ungraded part) which connects through a double-bored two-way stopcock (2) with either 3. Inlet tube (as shown) or 4. Outlet tube.

70 mm in length and closed by rubber stoppers at both ends. Tho lower stopper is pierced by the capillary tube and an outlet tube which only permits the surface of the surplus fluid to ascend to within some millimeters of the capillary tip. In the upp stopper is fitted a short glass tube, bent right angles. A short puff in this tube w empty the chamber (touching with the li is unnecessary). Backflow through the cap lary is impossible (see Fig. 1.) even if empt ing is inadvertently omitted. If preferred th device may be replaced by a short glass tu and a rubber or plastic tube running t container for constant drainage.

The burette is flat and sturdy and cons quently easily and sefely packed. Clampi quently easily and sefety packed. Clampi around the overflow-chamber and lower u graded part of the burette tube (protect with a piece of rubber tubing) involves danger of breakage. The price will be re sonable on account of the simple design a when the stopcock begins to leak because wear the replacement is easy. Cleaning not difficult because of the straight a strong upper tip. In practice the overflo through the thin capillary is scant and cons quently may be discarded.

ACKNOWLEDGEMENT

The firm F. C. Jacob Glasteknik, Copenhag is thanked for excellent workmanship.

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QUANTITATIVE DETERMINATION OF FIBRINOGEN IN PLASMA GRAM'S METHOD MODIFIED

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)uring the past few years the interest in inogen determination has increased, not t in obstetrics after the discovery of the o- or afibrinogenemia as a cause of gross ding in parturition.

Iost of the methods are rather time-con- ing, which does not matter when the inogen determination is made in medical s (jaundice, hemorrhagic disease). But bleeding in some obstetrical emergency ɔncerned, it is necessary to have a rapid hod giving the result in minutes instead ıours.

ı Denmark the gravimetric method of m is in common use; this method has a ı accuracy, but the procedure takes a ér long time, 5—6 hours. Recently Kors- rd Christensen (1955) has published a ıod using trypsin degradation of the clot photometric determination of the amino thus formed. The method takes about ɔurs, but may be accelerated to about 1½ r without serious loss of accuracy.

he different steps of Gram's method e been revised and an attempt to accele- them has been made with the aim of not ıg any of the accuracy.

METHOD

Principle

ıe fibrinogen of oxalated plasma is converted ɔrin by addition of thrombin. After drying, the is determined gravimetrically.

Procedure

Blood sampling: A centrifuge tube with a at 5 ml is prepared with *dry oxalate mixture* g oxalate mixture per 1 ml blood; oxalate mixture being 1.2 gm of crystalline ammonium oxalate and 0.8 gm of crystalline potassium oxalate. The salts are dissolved in distilled water and made to a final volume of 100 ml. 0.5 ml anticoagulant, subsequently dried, is used for each sample of 5 ml blood). Venous blood to the 5 ml mark. The coagulation is prohibited by inverting the tube.

2. Centrifugation for 2—3 min. at 3000 R.P.M.

3. To a test tube with flat bottom, 100 × 26 mm, containing 5 ml of 0.9 per cent sodium chloride is added 1 ml plasma. Mixing.

4. 0.2 ml trombin solution containing 100 N.I.H. units thrombin per ml is added. Thorough and rapid mixing. The clotting is complete in 2 minutes. The thrombin solution ("Thrombin, topical", Parke, Davis and Co.), 100 N.I.H. units per 1 ml of 0.9 per cent isotonic saline, is divided into aliquots of about 0.5 ml in rubber stoppered smal test tubes. The solution will keep at —10—20° C for months.

5. The clot is loosened from the walls of the tube by shaking the tilted tube, and transferred to the middle of a pile of 6—8 pieces of filter paper, preferably of a sort with a rather smooth surface, (*e. g.* Munktell No. 0, 11 cm). Then another pile of 6—8 pieces of filter paper is placed on top of the clot, and a gentle pressure is exercised by placing a 200 ml Erlenmeyer flask with water on the pile. In 5, seldom 10 minutes all fluid has been sucked out of the clot which now sits on the surface of the inmost filter paper as a glistening membrane. The membrane is cautiously loosened from the filter paper by means of a needle or a stiletto. Care must be taken not to tear the edge of the membrane.

6. The clot is rinsed in 0.9 per cent saline for some seconds, then in 99 per cent alcohol for 5 minutes and finally in ether for 5 minutes. If a rapid answer is required the clot may now be weighed, if not, the clot is incubated at 37° C for 2 hours and then weighed.

7. Calculation: Weight of clot in mg × 100 = mg fibrin(ogen) in 100 ml plasma.

In case of severe hypofibrinogenemia, the clot which is formed after adding thrombin is so fragile that it is not possible to remove it without tearing

it after drying on the filter paper. It is easy to recognise before removing the clot from the tube that the fibrin content is very low, the gelatinous clot being only slightly turbid and almost fluid when the tube is tilted. In this case the entire clot is taken into a centrifuge tube and centrifuged for 5 minutes at 3—4000 R.P.M. Now the fibrin clot has separated somewhat from the fluid and can be removed with a clean tweezer or needle as a tiny ball which is then placed on filter papers and dried in the usual way. The drying procedure will take only a couple of minutes. It is recommended not to place filter paper on the top of the clot in this case. The membrane thus formed from the clot is only a few mm^2 in size and can easily be loosened for the rinsing procedure.

Weighing the clot immediately after the ether rinsing (and after the ether has evaporated which takes only a few seconds) gives values averagely 5 per cent higher than after 2 hours of drying at 37° C.

The whole procedure takes about ½ hour; if the highest accuracy is required 2 hours for drying must be added.

DISCUSSION

The procedure differs from the original Gram's method in two important steps, namely blood collecting and coagulation. Gram uses 0.5 ml 3 per cent sodium citrate solution and 4.5 ml blood. He states that this anticoagulant is osmotically inactive so that fluid does not pass to or from the red blood cells changing the plasma amount. In order to calculate the fibrin content in plasma he uses the formula

$$\frac{A \times 100 \times \text{ml citrate}}{\text{ml plasma}}$$

where A is the weight of the fibrin in mg.

This calculation is somewhat lengthy, requiring centrifugation for 1 hour, and not entirely accurate, because it is almost impossible to have the blood cells thrown completely down without any plasma between them.

In the modification of Gram's method these difficulties have been overcome by using the dry oxalate mixture (ammonium and potassium oxalate) which is osmotically inactive. After centrifugation for a few minutes the supernatant layer is plasma and no calculation is necessary.

The coagulation after Gram's method is established by recalcifying the plasma. The procedure — in order to become complete — takes a rather long time, 1½ hour, at 37° C. In the modified method, a thrombin preparation is used which establishes the complete coagulation in 2 minutes at room temperature.

The removal of fluid from the clot is accelerated somewhat in the modified procedure by placing filter paper both under and above the clot and further by exercising some pressure on it.

It is a draw-back — in case of severe hyprofibinemia — that the clot is so fragile and tiny that is is not possible to dry it in the usual manner. Yet it is possible for the skillful technician to recognize when the specimen originates from a case of hypofibrinemia by the appearence of the clot alone because of its lack of turbidity and its relative fluidity. So she will know, at this step of the procedure, that a centrifugation is necessary before drying the clot on filter paper.

The after-drying after rinsing in alcohol and ether is only necessary when the highest accuracy is needed. In 20 cases the clots were weighed after the rinsing and again after 1 and 2 hours of drying at 37° C. The weight had decreased on the average 2.5 and 5 per cent respectively. Further drying at 37° C gave no further decrease of weight. The weighing was carried out on an analytical balance allowing readings of tenths of a milligram (Mettler balance).

Table I. *Gram's method compared with the modified method.*

Gram's method	Modified method	Difference per cent
314	320	+ 2
279	310	+11
235	270	+ 7
240	240	0
261	260	0
276	270	— 2
317	310	— 2
322	330	+ 2
288	310	+ 8
300	290	— 3
		+ 2.5

ıe modified method has been compared the original Gram's method (Table I). concordance of the two methods is good, alues of the modified method being on verage slightly (2.5 per cent) higher.

ıe plasma fibrinogen level has been ıined with the modified Gram's method 3 healthy males and 28 healthy females, ly young adults. The sedimentation rate ıe red cells was in all cases normal. The ı values were almost identical in the two s. The mean for all the 51 persons was mg per 100 ml, with the scattering —410 mg per 100 ml. The standard ıtion was found be 50.7. The normal es ($\pm 2\sigma$) are 216—419 mg per 100 ml. ıe normal values agree well with those ıe literature.

ACKNOWLEDGEMENT

ıe skillful assistance of Miss Bodil Rasmussen knowledged.

SUMMARY

The determination of the fibrinogen level of plasma by Gram's method has been modified into a more rapid but not less accurate method which gives the result in about half an hour, Gram's method taking about 6 hours.

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Scandinav. J. Clin. & Lab. Investigation 208—209, 9, 1957.

A PORTABLE SOURCE OF WOODS LIGHT IN PORPHYRIN ANALYSIS

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The red fluorescence of porphyrins is well known and extensively used in porphyrin analysis, but it is not generally realized that it is so inexpensive and easy to study that it is within the reach of every laboratory. One of the pioneers of porphyrin studies (Waldenström, 1937) writes: "Natürlich ist der Nachweis einer solchen Fluoreszenz sehr einfach, er fordert aber eine spezielle und kostbare Apparatur, die eigentlich nur für diesen Zweck verwendbar ist." But this is no longer true as today the luminous discharge lamp has grown from a laboratory fitting to a commercial article (Radley & Grant, 1954).

In laboratories possessing a source of Wood's light this is most often fixed in some place, usually a dark room or a dark case on the wall in a remote corner, and thus the analyst has to walk a certain distance every time a fluorescence test is required — to decide whether an extration is completed or if sufficient material has been placed on a paper chromatogram *etc.* This may amount to several miles a day in a laboratory with many porphyrin analyses and can be avoided by placing a portable source of Wood's light on the bench. If the fluorescence is not too weak it can be detected in semi-darkness, and real darkness is only required when the porphyrin amounts are very small.

The light is not harmful to the eyes as is the case with Hg-quartz light. Wood's light from a lamp on the bench has been employe in our laboratory for three years without a harmful effects. Further, our lamp has be transported by car or by train for lo distances and tolerated this, and it is thus remarkably robust piece of laboratory equi ment. Its price is reasonable as a lamp co sisting of an Osram HQW 500 bulb and t corresponding special transformer only cos 150—200 Danish Kroner. The bulb is co veniently mounted with the transformer foot, and this can be done by any electri technician or hospital workshop. The tran former may also be placed hanging from t ceiling and the bulb as a hanging lamp is often used in laundries where Wood light is employed to read writing on lin with invisible ink. The only indispensib requirement is alternating current.

Such portable lamps may be useful *several branches of medicine.* Although p phyrias are regarded as rare diseases, e dence is accumulating which shows that least acute and chronic porphyria — togeth forming the hepatic porphyria of Watson cannot be very unusual, and the need rapid diagnosis may arise in surgical as w as in medical, psychiatric and dermatologi departments. Further, it is worth emph sizing that congenital porphyria in swine a cattle often exists in clinically latent for which can only be diagnosed on inspecti of teeth or bones in Wood's light, and t these slight cases are much more numero that the clinically manifest more severe o (Jørgensen & With, 1955; With *et*

[1] Supported by a grant from The General Research Foundation of the Danish State.

7). And for this reason I believe that genital porphyria in man may exist as a nt disease much more frequently than ved at present.

he *morbid anatomist* may use Wood's t to verify or exclude the diagnoses of genital or chronic porphyria (*porphyria nea tarda*) on examination of bones or n. And by routine studies of bones in e series of autospies, the frequency of nt cases of this condition in the popula- can be studied. In such a series exami- on of a piece of a rib or the vertebral mn would be sufficient.

he *dermatologist* would be interested in diagnosis of porphyria in light dermato- of the types *hydroa aestivale* and *hydroa iniforme*. Here examination of the teeth ne patients in Wood's light might be help- As the porphyrins are deposited primarily ne dentin and cement one has to look for fluorescence where the enamel is thin or nt. A systematic study of the fluor- nce of the teeth of such patients would asy to perform with a portable source of od's light.

acteriologists may find the lamp useful use many microbial species are known produce porphyrins. Demonstration of phyrin in cultures become simple and with a transportable lamp and may be alue in differentiation of various strains nicroorganisms.

he branch of medicine most interested he lamp is, however, *chemical pathology*, as it is inexpensive and easy to handle *ught to be available in every hospital ratory*. From the laboratory it may be sported to the other departments when uired.

pplications in *biological studies outside medi-* are briefly mentioned. The red fluorescence he feathers of owls, goatsuckers, and bustards (Völker, 1938) as well as of the egg-shells of several birds — *e.g.*, the domestic hen — and the shells of many molluscs, is well known, but this phenomenon has not been *studied on living animals*. This is made possible by the portable source of Wood's light. Owls (*Athene noctua* and *Syrnium aluco*) exhibit a very beautiful scarlet color of the flanks and the inner surface of the wings when seen in darkness or semi-darkness in Wood's light, and a common hen's egg of brownish color has a deep purple fluorescence.

SUMMARY

The portable source of Wood's light is inexpensive and robust and ought to be found in every hospital laboratory. It is a valuable aid in the diagnosis of porphyria in the laboratory where it may save time, and it can also be used in the departments to study the fluorescence of teeth on living patients and of bones in the autopsy room. Its application in the study of cultures of micro-organisms and of living animals such as owls is mentioned.

ACKNOWLEDGEMENTS

I am indebted to Hospital Engineer K. J. Hansen for generous help in the construction of the portable lamp. Further I want to thank professor R. Spärck, D. Sc. of Copenhagen University and Holger Poulsen, D. Sc. of the Copenhagen Zoo for generous help and cooperation.

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Letters to the Editors

Scandinav. J. Clin. & Lab. Investigation 210, 9, 1957.

QUANTITATIVE DETERMINATION OF CHOLESTEROL IN SERUM WITH P-TOLUENE SULPHONIC ACID

We were interested to read Dr. V. Leppänen's (1956) conclusion that the p-toluene sulphonic acid method for the quantitative determination of total cholesterol in serum was suitable for routine analysis.

We have, since early 1956, employed the procedure described below, as a method for checking the serum cholesterol alterations observed in fat feeding experiments.

Good agreement with the values as determined by the Anderson & Keys modification (1956) of the Abell, Levy, Brodie & Kendall methods (1952) has been obtained.

The procedure we employ is simple, precise and reproducible and consists of an extraction procedure based on that of Drekter (1943) followed by the colour reaction of Pearson, Stern & McGavack (1953).

We feel that this procedure, which requires neither special apparatus nor elaborate control of conditions of colour development, may be of value to the clinical chemist in the normal hospital laboratory.

METHODS

Reagents

Acetic anhydride.
Glacial acetic acid
12 per cent p-toluene sulphonic acid in glacial acetic acid.
Standard solution of cholesterol: 80 mgm per cent cholesterol in glacial acetic acid.
Sulphuric acid concentrated.

Procedure

Sera: Into a stoppered centrifuge tube pla 0.2 ml of serum. Add 0.5 ml glacial acetic aci mix, then add 5.0 ml acetic anhydride. Rotate t mix then after 5 to 15 mins centrifuge at 2000 RP for 10 mins. Transfer 3.0 ml of supernatant flu to a colorimeter tube add 1.0 ml p-toluene sulphon acid solution and allow to stand for 30 mins. Ad 0.4 ml sulphuric acid. Mix well. Read in phot electric colorimeter at 550 mμ after 20 mins. Th colour is stable for a further 10 mins.

Blank: use 0.2 ml water in place of seru

Standard: water 0.2 ml + standard solution cholesterol 0.5 ml. Proceed as for sera but omittin the 0.5 ml of glacial acetic acid.

Calculation:

$$\frac{V - B}{S - B} \times 200 = \text{mg per cent.}$$

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February 1957.

T. J. Turner,
L. Eales.

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CREASED CONTENT OF A δ-AMINOLAEVULIC ACID-LIKE SUBSTANCE IN URINE FROM WORKERS IN LEAD INDUSTRY

is known that coproporphyrin as well as educed precursor is excreted in increased ants in lead poisoning. The metabolic der responsible for the changed por- in metabolism is obscure. No data are able to suggest any abnormal metabolism orphobilinogen or uroporphyrin I or III.

We have studied the excretion of δ-aminolaevulic acid, the precursor of porphobilinogen, in industrial workers exposed to lead. The workers are regularly examined for hematologic changes and for increased excretion of coproporphyrin in order to trace any lead poisoning as early as possible. At

se	Age in years	Exposed to lead in years	Blood picture			Urine analysis		
			Hemoglobin g/100 ml	Red blood cell count. mill.	Basophilic stippled cells/10000 red blood cells	Urobilinogen	PBG μmol/ml x10³	ALA μmol/ml x10³
B.	40	20	16.2	5.3	2	+	6	267
F.	44	26	15.2	4.8	24	0	0.6	33
L.	45	31	16.0	5.0	0	(+)	6	56
.	52	30	12.3	4.1	14	0	3	110
.	48	28	16.0	5.0	21	0	3	190
.	60	34	14.9	4.8	5	(+)	3	78
.	57	34	16.5	5.1	6	(+)	3	19
.	59	5	15.4	4.8	0	(+)	7	55
M.	54	5	15.4	4.6	2	(+)	7	36
M.	42	7	14.7	4.6	4	0	2	81
M.	29	5	16.0	5.0	3	(+)	5	27
M.	51	31	16.0	4.9	0	(+)	6	186
N.	23	1 3/4	16.3	5.0	12	0	4	42
N.	49	31	15.8	5.1	9	(+)	4	52
O.	38	21	15.8	5.0	2	0	2	45
O.	54	4	14.2	4.6	0	(+)	5	55
O.	37	19	15.7	4.9	7	0	1	27
P.	55	21	13.6	4.4	13	0	0.6	243
P.	55	33	14.4	4.7	10	(+)	2	47
T.	40	1	13.9	4.8	0	(+)	4	29

the time of the investigation none of the workers had any clinical symptoms or signs of lead poisoning.

Porphobilinogen (PBG) and δ-aminolaevulic acid (ALA) in the urine were determined quantitatively according to Mauzerall and Granick (1956).

The material and the results of the analysis are summarised in the Table. It might be added that none of the urinary samples showed any evidence of albuminuria, glycosuria or increased amounts of coproporphyrin. The control material consisted of urine from 60 adults. The mean value of the PBG excretion was 0.0028 (S.D. 0.0015) μmoles per ml. and of the ALA excretion was 0.0165 (S.D. 0.0086) μmoles per ml. Thus only 4 of the 20 lead workers showed a slightly increased excretion of PBG, t 15 showed an increased excretion of AL and in 10 the increase was striking. It however, not yet certain whether the me bolite determined by the method of M.. & really always must be ALA. This possibili is receiving attention.

The observations presented above sugg that *any influence of exposure to lead in many cases, demonstrable earlier by th method than by determination of copropo phyrin or haematologic changes.*

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Malmö, Sweden.
April 27, 1957.

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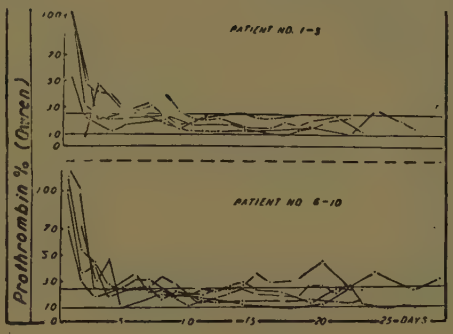

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Litteratur.

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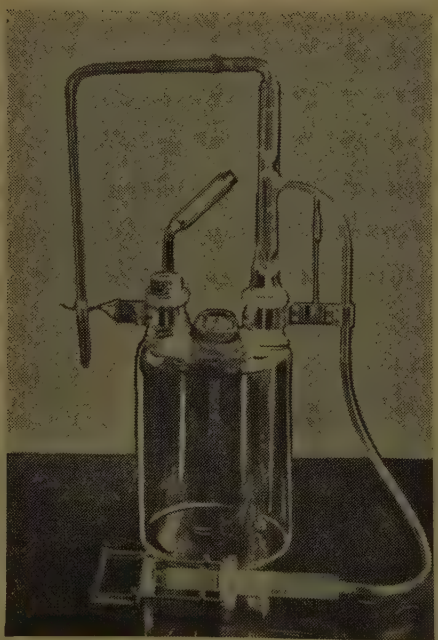

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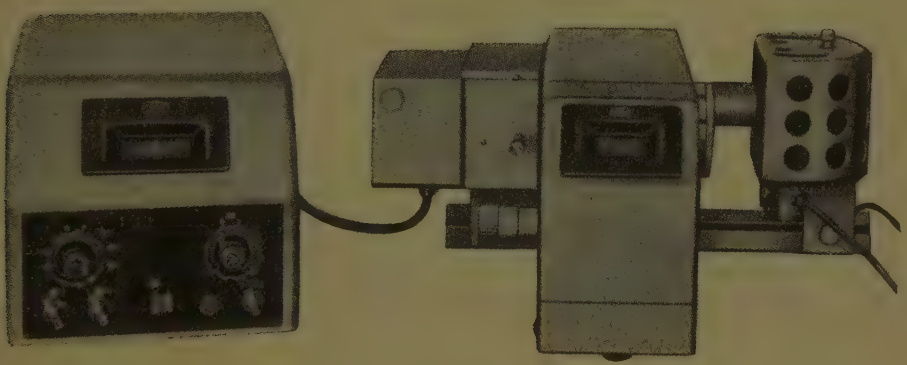

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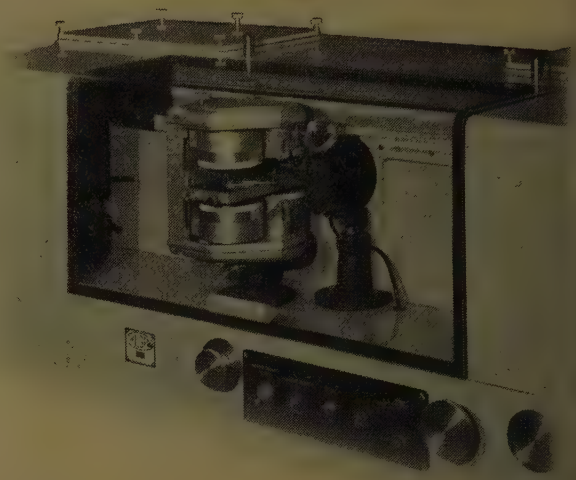

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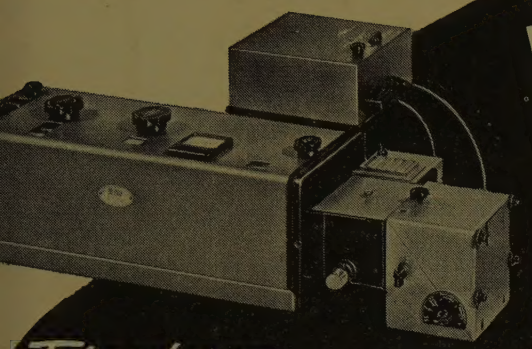

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